Vol. XXIII. *August, 1912.* *No. 3*

BIOLOGICAL BULLETIN

THE INTERSTITIAL CELLS AND THE SUPPOSED INTERNAL SECRETION OF THE CHICKEN TESTIS.[1]

ALICE M. BORING.

The problem presented in this paper was suggested to me by Dr. Pearl in connection with some work of his on the time when the secondary sexual characters first appear in the domestic chicken. The domestic chicken has a long list of secondary sexual characters. Will the cytology of the reproductive organs throw any light on the cause of the development of these secondary characters? The observations described in this paper deal with the cytology of the testis, with especial reference to the problem of secondary sexual characters.

The problem of the cause of the secondary sexual characters has been approached in many ways, but the favorite one is the internal secretion theory, that is, that the secondary sexual characters are dependent on a secretion formed by the cells of the primary reproductive organs. Some phases of this theory consider the germ cells themselves as the source of this secretion, but oftener the so-called interstitial cells are regarded as the secreting agents. We find frequent references to these interstitial cells in the literature, but there has been great confusion between the terms "interstitial tissue" which might be any connective tissue lying between the seminal tubules, and the term "interstitial cells," which by some workers is used for gland cells among the connective tissue between the tubules. Workers disagree as to whether these gland cells are epithelial or mesenchymous in origin. Another term frequently used is "interstitial gland" which seems to be used for any large accumulation of the

[1] Papers from the Biological Laboratory of the Maine Experiment Station, No. 38.

gland cells in the interstitial tissue. Poll, in his studies of hybrid birds, speaks of the increased development of the interstitial gland composed of cells of epithelial nature in contradistinction to the connective tissue. Ceni, in his account of experiments with feeding ducks on caffeine and removing the cerebrum, speaks of an increase of the amount of interstitial tissue at the expense of the cells of the seminal tubules and mentions groups of epithelial cells called interstitial glands. On the other hand, Loisel distinctly states that he finds no evidence of activity in the cells of the interstitial tissue of birds at any time of year, but finds a fatty secretion formed by the Sertoli cells. Mazzetti finds no more evidence of secretory granules in the cells of the interstitial tissue than in the cells of the seminal tubules. He claims that interstitial cells are derived from connective tissue cells and gives an interesting list of variations in the number of interstitial cells in different animals and in the same animal at different times of year. Kirkbride states that the number of interstitial cells in the testes of newborn infants is very variable. Gudernatsch describes a case of hermaphroditism in man where the interstitial tissue of the testicular portion of the ovotestis was enormously developed, and yet the secondary sexual characters were such that the individual was usually regarded as a female.

So the question of the origin, nature and function of the interstitial cells has been by no means settled. Now if it is a general law that the interstitial cells and secondary sexual characters are causally interrelated, one would certainly expect to find abundant interstitial cells in a bird with such marked secondary sexual characters as the domestic chicken.

Methods.

Just-hatched chicks were used first, as there are some indications that there may be a differentiation in respect to certain secondary sexual characters at that early age. The chicks were all Barred Plymouth Rocks from the Maine Experiment Station stock, not a day old. The gonads are easily cut off from the surface of the kidney with scissors. They were fixed in Gilson's, Flemming's and Hermann's solutions, these two osmic

agents being used especially because the internal secretion of the testis has usually been described as appearing in the form of fat or oil drops in the cells.

For comparison with these, I have studied the testes of fourteen older birds, ranging from 5 to 12 months in age. These were all but three pure Barred Plymouth Rocks, one was a White Leghorn, and two were cross-breds. They were killed at different times of year, October, November, January, March and April. In birds over six months of age, dividing germ cells were found, all stages from spermatogonia to spermatozoa. The testes from six of these birds had been put up previously for other purposes for which the best cytological methods were not necessary, but the general observations on these accord so well with those on the very best fixed material, that I do not hesitate to state that my observations cover fourteen birds. The other eight testes were cut in small pieces and fixed in Gilson, Flemming and Hermann. With two birds, every possible precaution to secure absolutely normal fixation was taken: the testis was removed from the living bird and put directly into Flemming, heated to the temperature of the bird's body, and the testis was then cut into thin slices with a razor, as it was held in the hot Flemming.

The ordinary method of clearing in xylol for embedding and mounting was not used with all of the material, as xylol dissolves fat. From Mallory and Wright's "Pathological Technique," I found that chloroform and clove oil do not dissolve fat fixed in osmic acid, so by clearing the Flemming material in chloroform or clove oil, and mounting the sections in chloroform balsam, I got preparations showing fat.

Observations.

In sections of the just-hatched testis, one can see without great magnification that about half the substance is interstitial connective tissue (Fig. 1). The seminal tubules are small and far apart. Careful detailed study shows that the cells of the seminal tubules have comparatively large round nuclei, with a linin network and scattered chromatin granules; in fact, they are typical resting germ cells. There were no signs of any

dividing cells, or of any spermatocytes or spermatozoa. These cells are evidently quiescent early spermatogonia.

The abundant interstitial substance shows no marked differ-

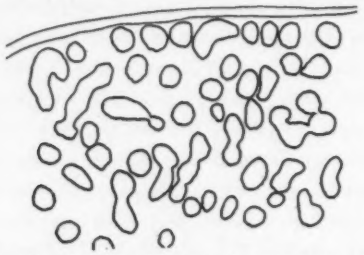


FIG. 1. Section through testis of just-hatched chick, showing seminal tubules in outline, interstitial spaces, and testis sheath. × 100.

entiation. It looks like embryonic connective tissue (Fig. 2). The fibers run in all directions. The nuclei are mostly round, but some of them are elliptical or irregular in shape. However,

FIG. 2. Portion of Fig. 1, showing nuclei and fat (*F*) in interstitial tissue. *C* = blood corpuscles. × 1,000.

the nuclei in the connective tissue sheath of the testis are all typical elliptical connective tissue nuclei. Why this difference? One cannot help noticing that the texture of the interstitial

substance where the nuclei are mostly round is loose and reticular, while the fibers of the sheath lie parallel and close together, and look as though they were stretched tight around the testis. This suggests that the difference in the shape of the nuclei may be due to difference in pressure relations.

Further, none of these cells of the interstitial substance show any signs of glandular activity. The so-called "interstitial cells" have been described in a recent paper by Mazzetti as grouped in complexes in the intercanalicular spaces, as polyhedral or ovoid with abundant protoplasm, large round nucleus, an evident nucleolus, protoplasm in two zones, a clear peripheral and an intensely-coloring central, also as containing secretory granules, either crystalline or fatty in nature. There are no such cells in the interstitial substance of the testis of just-hatched chicks, and the connective tissue cells which make up this substance show no sign of glandular activity. In sections of material cleared in clove oil so as not to dissolve fat, great masses of fat stained black by osmic appear in the interstitial tissue, but they are not grouped about any especial cell, and have no appearance of being secreted by any of these cells (Fig. 2).

This being the condition in the quiescent testes of the just-hatched chick, we may next compare with it the testes of birds in which active spermatogenesis is going on.

In the older birds, there is a striking individual variation in the size of the seminal tubules. This variation has no relation to breed, age, time of year, or apparent stages of the germ cells, but occurs in birds of the same breed, and age, killed on the same day, with the germ cells in both showing all stages of spermatogenesis. However, there is one constant relation, the relation of the size of the seminal tubules to the amount of interstitial tissue. Wherever the seminal tubules are small the interstitial spaces are wide, and where the seminal tubes are large, the interstitial spaces are narrow. Figures 3 and 4 bring out this point. It is not only the width of the spaces between any two tubules that varies but also the size of the triangular spaces where any three or four tubules come into juxtaposition. This relation can be proved by accurate measurements.

For these measurements, I used slides from the testes of four Barred Plymouth Rock cockerels of about the same age, killed on the same day, all in active spermatogenesis. The measuring was done with an eyepiece micrometer. One hundred seminal

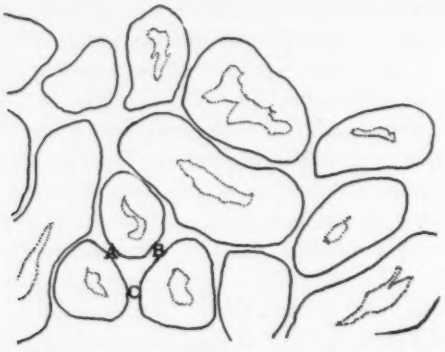


FIG. 3. Section of testis of ♂ 666, showing small tubules and wide spaces. × 100. *ABC* = one triangle in which the cells were counted, such as Fig. 5 represents.

tubules were measured in each of the four birds, and 100 spaces between tubules. There seems to be no accurate way to measure the triangular spaces.

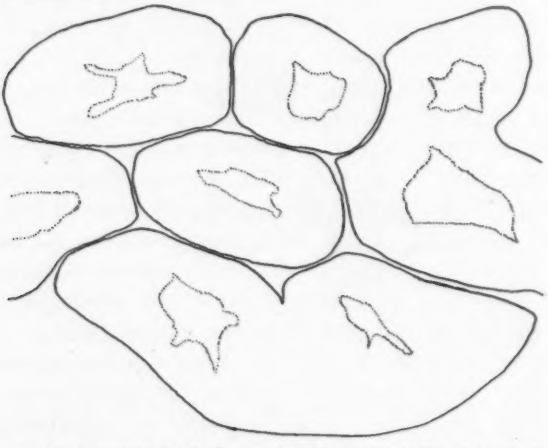


FIG. 4. Section of testis of ♂ 147, showing large tubules and narrow spaces. × 100.

A glance at Table I. will show the relation between the two sets of means: taking the birds in the order nos. 666, 1271, 2323, 147, the tube size increases, and the space width decreases.

TABLE I.

ABSOLUTE MEASUREMENTS AND CELL COUNTS OF TESTICULAR ELEMENTS. MEAN VALUES.

Bird No.	Tube Width.	Space Width.	No. Cells in Triangle.	No. of Long Cells.	No. of Round Cells.	No. of Light Cells.	No. of Dark Cells.	No. of Dark Long Cells.	No. of Dark Round Cells.
666	45.43	3.46	67.68	33.16	34.52	59.00	8.68	3.28	5.40
1271	50.82	3.015	60.76	27.32	33.44	58.92	1.84	.24	1.60
2323	82.99	1.455	23.16	14.92	8.24	20.60	2.56	.80	1.76
147	93.14	1.385	50.96	33.68	17.28	50.08	.88	.48	.40

TABLE II.

RELATIVE PROPORTIONS OF DIFFERENT CELL ELEMENTS.

Bird No.	Per Cent. Long Ce s.	Per Cent. Round Cells.	Per Cent. Light Cells.	Per Cent. Dark Cells.	Per Cent. Dark Long Cells	Per Cent. Dark Round Cells.
666	48.98	51.02	87.17	12.83	37.79	62.21
1271	44.96	55.04	96.80	4.20	13.27	86.73
2323	64.42	35.58	88.94	11.06	31.25	68.75
147	66.09	33.91	98.27	1.73	54.54	45.46

On examining the interstitial spaces in these four testes with greater magnification, there appears to be what one would naturally expect, a greater number of cells in the larger triangles and wider spaces. An accurate count of the cells shows this to be the fact. The cells in twenty-five triangles of each of the same four testes were counted. In order to count over comparable areas the cells were counted from the points of the triangle along the spaces between each two tubules to about half way to the next triangle (Fig. 3, *A*, *B*, *C*). The sections in the four testes were of course cut the same thickness. A study of Table I. shows that the number of cells varies with the width of spaces and inversely as the size of the tubules, with one exception. That is, arranged according to mean number of cells in spaces, the birds stand in the order 666, 1271, 147, 2323, as contrasted with 666, 1271, 2323, 147, the order for tubule size. The exception is in the reversal of 147 and 2323. The explanation of this lies in the fact that the measurements are of the spaces between two

tubules and the count of cells includes the triangular space between three tubules. There is very little difference in the space width of 147 and 2323, but the triangles of 2323 are decidedly smaller.

This difference in tubule size is so striking and seems so strange in birds so nearly alike in other ways, that I tried to find any possible other explanation than that of individual variation. It occurred to me that possibly the size of tubules might vary in different parts of the testes, and as the pieces of testes first sectioned were cut out of the organ without any reference to position the probability is that they were from different parts in the four different testes. However, in two birds killed, I carefully fixed pieces from the periphery and from the center of the organ, as these two regions would represent any difference in pressure conditions or in growth. The sections of these pieces

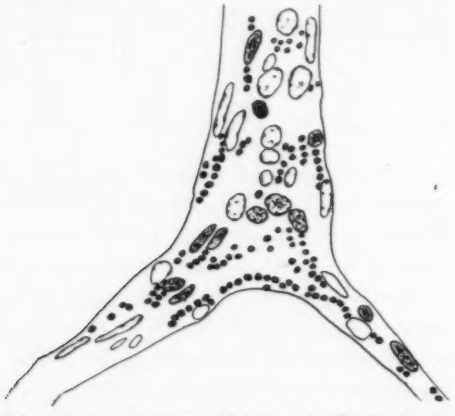


FIG. 5. Triangular space of interstitial tissue in ♂ 666, showing many nuclei of different shapes and staining capacity, also many fat globules arranged in rows. × 1,000.

show that the tubule size is the same throughout. Though the testis from which Fig. 3 is drawn has many fully formed spermatozoa, it is just possible that it has not reached the climax of growth, and would eventually have contained tubules as large as those in Fig. 4.

In the adult testis, the nuclei vary in shape exactly as in the

just-hatched chicks, and there are here also no cells differentiated from the connective tissue fibers. In the adult there are more elongated nuclei and fewer round ones. In the testis, where the tubules are large, and crowded close together, so that there is not much interstitial tissue between them, the nuclei are mostly elliptical, with some round ones in the triangle (Fig. 6). Where the tubules are small and far apart, leaving much interstitial tissue there are more round nuclei, and they lie not only in the

FIG. 6. Same as Fig. 5, but from ♂ 2323, showing few nuclei and few fat globules. *B* = blood vessel. × 1,000.

triangles (Fig. 5). In counting the nuclei as mentioned above, I differentiated between long and round ones. Table I. shows the mean number of each kind, and Table II. gives the percentage of each shape to the total number of nuclei. In ♂s 666 and 1271, with the small tubules, 48 per cent. and 44 per cent. nuclei are long, but in ♂s 147 and 2323 where the tubules are large, 66 per cent. and 64 per cent. are long. This relation must mean a difference in mechanical conditions of pressure.

If the difference in the cells of the interstitial spaces is in the shape of the nuclei, and the shape of the nuclei is merely due to mechanical causes, can we call any of these cells interstitial cells with the accepted meaning of the word? We could form a complete series of nuclei ranging gradually from the typical elliptical connective tissue nuclei to the round undifferentiated

nuclei (Figs. 2, 5, 6). From the figures and statements in Mazzetti's paper, it appears that he has made the shape of the nucleus the important factor in deciding which cells are interstitial cells. But his results, in entire agreement with those of the present paper, show a complete series of gradations from his so-called interstitial cells with round nuclei to typical connective tissue cells. Also he finds a variation in the proportion of interstitial cells in animals of widely different species. Here in the chicken there is as much variation in this regard in birds of the same breed, if we take the shape of the nuclei as the basis for deciding which are interstitial cells, as Mazzetti describes for different genera or orders.

The only other difference evident in the cells of the interstitial tissue is the staining capacity of the nuclei. This difference is

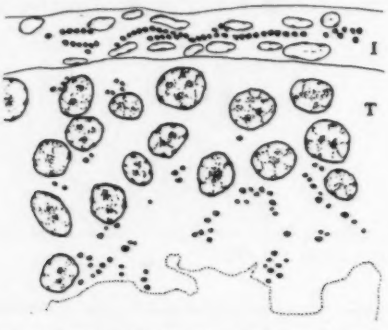


FIG. 7. Section of testis in ♂ 666, showing part of one seminal tubule (*T*) and the adjacent interstitial tissue (*I*). The fat is indicated by black dots. × 1,000.

not noticeable in just-hatched chicks, but very apparent in the older birds. Some stain very dark, and some remain almost unstained. Table I. gives the mean number and Table II. the percentage of dark and light cells. In ♂s 666, and 1271, there are 12 per cent. and 4 per cent. dark cells, and in ♂s 2323 and 147, there are 11 per cent. and 1 per cent. That would be a great variation in the per cent. of interstitial cells, if a dark staining nucleus could be regarded as a distinctive character of an interstitial cell. But it cannot. I made a differential count of the long and round nuclei among the dark staining ones, and found

37 per cent., 13 per cent., 31 per cent. and 54 per cent. of the dark-staining nuclei which have the long shape of typical connective tissue nuclei. It seems then that staining capacity of nuclei is not a satisfactory method of distinguishing interstitial cells.

There is one more point in the frequent descriptions of interstitial cells to be considered. Is there any evidence that the cells of the interstitial tissue are secreting fat? We have already found fat present in testes of just-hatched chicks, but decided that it probably was not a secretion. Fat is also present in

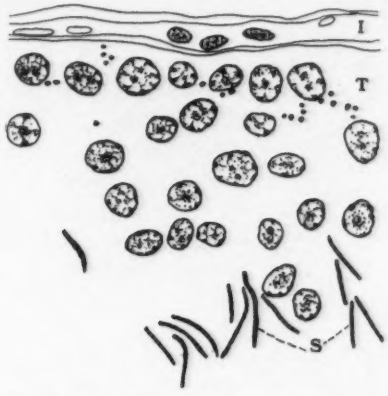


FIG. 8. Same as Fig. 7, but from ♂ 2323. *S* = spermatozoa. × 1,000.

the older testes, and is represented by the black dots in the drawings. Comparison of Figs. 5 and 7 with Figs. 6 and 8 will show that the fat is inside of the tubules as well as in the interstitial tissue. The fat globules in the interstitial tissue are not arranged like secretory granules in the cytoplasm of cells. They are mostly in long rows as though packed in spaces between connective tissue fibers. Figs. 5 and 7 show nearly as much fat near typical connective tissue nuclei as near the round or dark-stained nuclei of the "interstitial cells." A comparison of Figs. 7 and 8 shows much fat in Fig. 7 where there are only connective tissue nuclei in the interstitial space, and no fat in Fig. 8, where there is a small group of round dark-staining nuclei. Fig. 9 shows a part of a large mass of interstitial tissue where the nuclei are mostly round. This is evidently a so-called interstitial gland.

If interstitial cells or cells of interstitial connective tissue are secreting the fat found in the testis we certainly would expect to find it abundant in such an "interstitial gland," or if we prefer not to use that term, in so large a mass of interstitial tissue. However, there is not so much fat as in some of the smaller triangles and narrow spaces. This study shows *no evidence that the fat in the active testis is formed by the interstitial cells.*

FIG. 9. Part of an " interstitial gland " in ♂ 666, showing many round nuclei, but comparatively little fat. × 1,000.

It seems likely that this fat is being brought to the testis by the general metabolic processes possibly in connection with sexual activity, just as fat is deposited in the yolk of eggs in the hen. This would fit in with the former suggestion that possibly the testis in ♂ 666 is not as far developed as 147 and 2323. The tubules in 666 are smaller and the quantity of fat is greater. It may be in the process of active growth, while 2323 has reached the climax of growth of tubules, and used up most of the fat for this growth.

SUMMARY.

In conclusion, then, I find no cells in the interstitial tissue in the young or old chicken testis with the cell bodies differentiated from the connective tissue fibers. No evidence has been found that differences in shape of the nuclei are indicative of functional differences in the various cells of the interstitial tissue. On the

contrary, it appears that these differences in shape depend on mechanical pressure conditions. The difference in the staining capacity of the nuclei is not a basis for cell classification, for the nuclei which take a deep stain include those of all shapes. The fat in the testes is probably not formed by the interstitial tissue at all, but is brought there by the circulation and deposited. So we are brought to the conclusion that there are no "interstitial cells" in the testis of the domestic chicken in the sense that this term has been previously used. Furthermore no evidence has been found in this study which would indicate that an internal secretion of any kind is formed by any cells of the interstitial tissue. This result would appear to derive interest and significance in consideration of the fact that in few animals is there so extensive a development of secondary sexual characters as in the male of the domestic fowl.

LITERATURE.

Ceni, C.

'09 L'influenza del Cervello sulla sviluppo e sulla funzione degli organi sessuali maschili. Riv. sper. di Freniatria. 35.

'09 L'influenza di alcune sostanze d'azione prevalentemente cerebrale sulla funzione dei testicoli (Caffe, Veronal, e Absinthe). Riv. sper. di Freniatria., 35.

Gudernatsch, J. F.

'11 *Hermaphroditismus verus* in Man. Am. Jour. Anat., 11, p. 267.

Kirkbride, Mary B.

'11 Embryogenic disturbances of the testis. Arch. f. Entw. Org., 32, p. 717.

Loisel, G.

'03 Élaborations graisseuses periodiques dans le testicule des oiseaux. Comp. Rend. de l'Association des Anat., 5th Session, Liege, p. 222.

Mazzetti, L.

'11 I caratteri sessuali secondari e le cellule interstiziali del testicolo. Anat. Anz., 38, p. 361.

Poll, H.

'06 Der Geschlechtsapparat der Mischlinge von *Cairina moschata* ♂ und *Anas boschas* ♀. Sitzber. der Ges. Nat. Fr., Nr. 1, p. 4.

Poll, H., u. W. Tiefensee.

'07 Mischlingstudien: die Histologie der Keimdrüsen bei Mischlingen. Sitzber. der Ges. Nat. Fr., Nr. 6, p. 157.

REGENERATION AND REGULATION IN PARAMECIUM CAUDATUM.

FLORENCE PEEBLES, PH.D.

The investigations described here were begun in February, 1910, upon some pure lines supplied through the courtesy of Professor Jennings. The experiments were undertaken with the hope of determining how new races arise, and if possible, to produce by artificial means, a new race differing in size and perhaps in other characteristics from the parent stock.

A long series of experiments was carried on, testing the effect of various foods, and also subjecting the organisms to other changes in the environment. The conjugation of a large and a small individual was followed in order to see if the size of the ex-conjugants was modified by the inequality of the gametes. It was found impossible by any of these methods to produce a race with new characteristics.

In the winter of 1910–1911 a study of the regenerative and regulative power of single individuals and of conjugating pairs was undertaken. It was hoped that after the removal of part of the cytoplasm, and possibly some of the nuclear material as well, a small race might be produced. It soon became evident that the removal of the nuclear material was not possible, for no cell or fragment of a cell (unless cut while in the process of division) recovered after the nucleus was injured. Owing to the extremely small number of successful operations, and the alluring interest of various side issues which arose in connection with the work the main problem was neglected for a time and other experiments, which will be described in this paper, have been carried on at intervals up to the present date.

In November, 1910, in a paper read before the Cambridge Philosophical Society, Levin ('10) described very briefly some experiments on *Paramecium* in which he produced races without a micronucleus, and others where he found it possible to divide a living cell so that each fragment received a portion of the

meganucleus. The brevity of the paper makes it difficult to determine how this result was brought about. I have not been able to find a more complete account of these preliminary experiments.

In June, 1911, while working in Professor Boveri's laboratory in Würzburg, I made a series of experiments on large races of *Paramecia* from Munich and Würzburg, and up to May, 1912, these pure lines were used for comparison with material collected in Bryn Mawr. During the course of my investigations a paper appeared by Calkins ('11) in which he describes in detail the behavior of fragments of *Paramecia* after the removal of some of the cytoplasm. My results confirm those of Calkins so exactly that I shall omit a full description of the individual experiments merely giving a summary of the results in tabulated form.

It gives me pleasure to have this opportunity to express my gratitude to Professor Boveri for the many courtesies extended to me during my stay in Würzburg, and to Professor Jennings for his kindness in supplying me with some of his pure lines.

Methods.

The methods followed were practically those used by Calkins except that instead of treating the cells with neutral red before cutting them, the animals were quieted by placing them in a small drop of tap water thickened with quince seed. Immediately after the operation a few drops of culture fluid were added. All cultures were kept in hollow slides in a moist chamber. The culture medium was changed every twenty-four to forty-eight hours as conditions required. The infusion made from Timothy heads, according to Jennings' method, was the culture fluid most generally employed. A most satisfactory culture medium if rightly used is a .2 per cent. solution of Horlick's Malted Milk.

Experiments.

A. *The Behavior of Cells after Removal of Part of the Cytoplasm.*

Thousands of *Paramecia* were cut during the course of these experiments. The fragments were examined four hours after the operation and then again on the following day. All fragments

living less than twenty-four hours after the operation were omitted from the records. By this process of elimination the making of tables was greatly simplified.

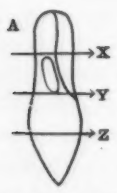


FIG. 1. Diagram of vegetative cell showing regions of the cut for removing the anterior or posterior end and dividing the cell in half.

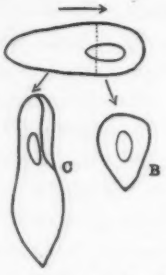


FIG. 2. Fragment of cell from which the anterior end was removed. B and C, the cells formed from it by division in the original plane.

1. *Removal of the Anterior End.*—In this experiment the anterior end of the cell was cut off at the level indicated in Fig. 1, *X*, thus removing from one fourth to one third of the cytoplasm. The small non-nucleated piece (Fig. 1, *A*) anterior to the cut usually disintegrates immediately after the operation, but in a few cases it closed in, forming a spherical mass which swam about rapidly for a day and then died. Such pieces never divide or show any sign of growth. The removal of the end often produces great disturbance in the larger nucleated piece. This is probably due to the fact that the macronucleus lies so near the level of the cut, and the oral groove prevents the closure of the wound. The following table gives the results from eighty-three individuals.

TABLE I.

Number of Paramecia.	Regeneration Followed by Normal Fission.	Division in Original Plane.	No Division.	Monsters.	Killed.
83	28	42	8	4	2

Thirty-four per cent. of the pieces developed new cilia at the cut end and grew to the normal size before dividing, but nearly fifty per cent. divided in the original plane (represented in all figures by the dotted line) forming a small irregular anterior

cell (Fig. 2, *B*) and a normal posterior cell (*C*). The smaller cell may grow rapidly to the normal size and divide into two equal cells, or it may form another small anterior and large posterior cell. At times the anterior cell finally produces a monster.

2. *Removal of the Posterior End.*—The removal of the posterior end of the cell through the region back of the mouth (Fig. 1, *Z*) produces less disturbance than cutting off the anterior end. This result would be expected as there is little danger of injury to the nucleus which lies in front of the cut. The results from this experiment are given in the next table.

TABLE II.

Number of Paramecia.	Regeneration Followed by Normal Fission.	Division in Original Plane.	No Division.	Monsters.	No Regeneration.
88	55	18	14	1	1

The percentage of fragments which regenerated and divided in the normal manner is much larger than that of Table I. Here sixty-two per cent. after recovery from the operation divided normally, forming a race of the original size, while only thirty-four per cent. of the cells in Table I. regenerated the lost end before dividing. In twenty per cent. of the fragments the division was irregular, resulting in a large anterior and a small posterior cell. This result stands out in sharp contrast to the fifty per cent. dividing irregularly in Table I. The probable explanation of this result is to be found in the fact that when the anterior end is removed the nucleus is either exposed directly to the surrounding medium, or is separated from it by a thin film of cytoplasm, and may be thus stimulated to divide in the plane already laid down before a re-arrangement of symmetry is effected. The result is that one daughter cell contains twice as much cytoplasm as the other, while both have the same amount of nuclear material. When the posterior end is removed (Fig. 1, *Z*) there is an equal amount of cytoplasm back and front of the nucleus, so that a new division plane forms, and when fission takes place each half gets an equal amount of cytoplasm. The later history of the small cells formed by irregular division will, I think, support this suggestion.

B. *The Behavior of Cells Cut in Half Transversely.*

It is extremely difficult to cut a *Paramecium* in half without permanently injuring both halves. The macronucleus almost invariably slips out and the pieces disintegrate within an hour or two. If, however, the nucleus is pushed forward or backward, as the case may be, into one of the halves that piece lives and will finally divide into two normal individuals. Out of forty cells thirty died without fission, and ten produced new races of the normal size.

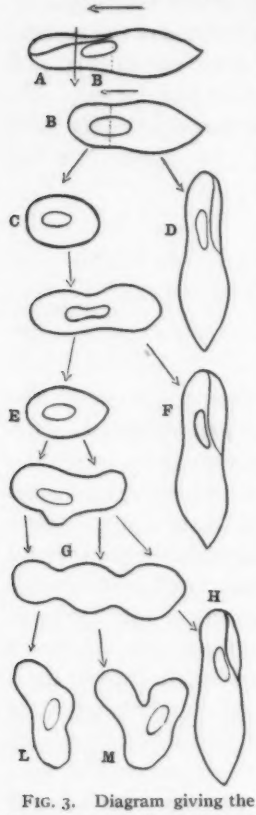


FIG. 3. Diagram giving the history of the formation of three normal races and two monsters from a cell from which the anterior end was cut off.

All of the experiments described so far were made, without exception, upon cells in the vegetative condition. None of the cells had recently divided or were preparing to divide.

C. *The Formation of Monsters.*

Calkins ('11) has shown that it is possible to produce from a small fragment a mass of such dimensions and structure that it represents many individuals, having several mouths, oral grooves, and vacuoles. I have not succeeded in obtaining more than a dozen of these monsters in the course of my investigations. Most of them developed during the warm weather in the latter part of June. Thinking that the temperature had something to do with their appearance I began a series of experiments under various degrees of warmth above that of the laboratory, but did not succeed in producing any more monsters than at room temperature. A brief description of several of these monsters may throw some light on the question of their mode of origin.

No. 1.—This monster arose from one of the fragments in Table I. where the anterior end of the cell was removed (Fig. 3). At the end of twenty-four hours the fragment (*B*) appeared active but showed no sign of fission; the cut end was slightly rounded. Before the end of forty-eight hours division took place in the original plane producing a small individual (*C*) and a large one (*D*). On the following day *C* began to divide. During the next night fission was completed and another small (*E*) and large (*F*) cell formed. On the fourth day *E* had increased in size, and showed signs of division. On the fifth day it had formed a chain of three cells (*G*), the most posterior of which formed a normal individual. The chain continued to throw off

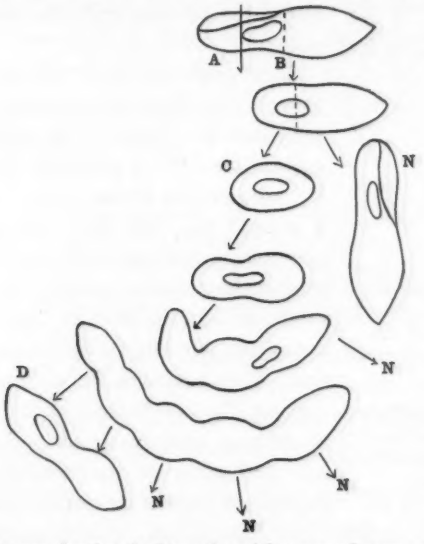


FIG. 4. Diagram showing the formation of five normal races and one monster.

normal cells posteriorly until finally after a period of growth a mass was left which separated into two parts (*L* and *M*). These when killed and stained were found to possess several mouths, but neither piece contained more than one nucleus.

No. 2.—The second monster arose in the same way from a small anterior cell produced by the division of a fragment in the original plane. In this case a chain of five parts (Fig. 4) formed

from the small piece (*C*). Finally the chain divided posteriorly into a series of normal individuals (*N*) leaving anteriorly one double cell (*D*). On the seventh day this monster was killed and stained. Three mouths, two vacuoles, and only one nucleus were present.

No. 3.—The third monster (Fig. 5) formed directly from the small anterior piece (*C*) without dividing. The piece grew rapidly for three days developing processes resembling budding individuals but none were thrown off. At the end of the third day the mass (*m*) was killed. Again only one nucleus was found but three mouths and four vacuoles were present.

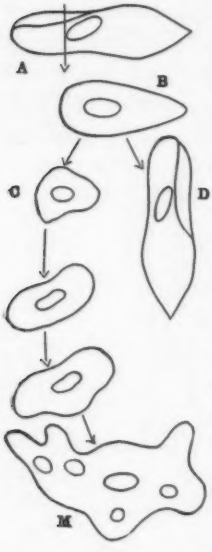


FIG. 5. Diagram showing the formation of a monster from a small cell without division.

Such cases as these demonstrate that there is great disturbance produced through the removal of some of the cytoplasm of a vegetative cell. The small piece (Fig. 5, *C*) contained all of the nuclear material for a normal cell, but less than half of the protoplasm required. Before the cell-body has attained its full growth the nucleus is ready to divide, and in turn when the cytoplasm is ready to divide the nucleus is not. This condition of affairs produces a complete loss of balance in the mechanism of division and the result is that irregular cells and monsters are formed and the cell rarely ever regains its normal condition.

D. *The Effect upon Cells Cut During Division.*

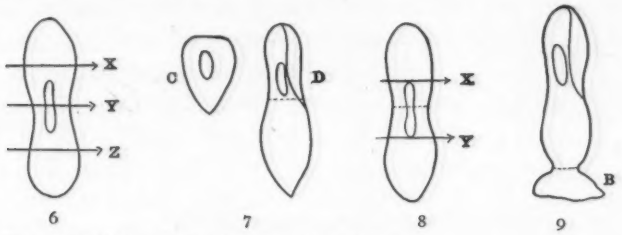
For these experiments *Paramecia* were selected which were in the early stages of fission, at a time when the nucleus was elongated and the body slightly constricted (Fig. 6).

Experiment 1.—When the cell is cut in half through the plane of division (Fig. 6, *Y*) both cytoplasm and nucleus are divided equally. The experiment is a comparatively simple one and is usually successful, both halves living and forming normal races.

The survival of both pieces depends largely on the stage of fission reached at the time of the operation. The nearer the process is to completion the more likely is the recovery of both halves.

Experiment 2.—When the cut was made through the center of the anterior or the posterior half of the dividing cell (Fig. 6, *X* and *Z*) the small non-nucleated piece always died, and the large piece continued to divide in the original plane forming a small cell (Fig. 7, *C*) and a large cell (*D*).

Experiment 3.—When the cut is made in either half of the dividing cell near the plane of division (Fig. 8, *X* and *Y*) the



6 7 8 9

FIG. 6. Dividing cell showing the region of the cuts.

FIG. 7. Small and large individual formed by division in the original plane after removal of the anterior end of a cell during the process of fission.

FIG. 8. Diagram showing cuts made near the center of a dividing cell.

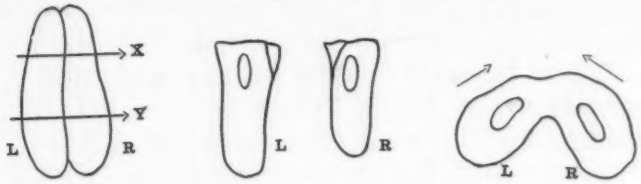
FIG. 9. Cell with fragment of the posterior half still attached.

small fragment (Fig. 9, *B*) remains attached to the other half, and is gradually absorbed. In a few cases division continued in the original plane, but this occurred only in those cells where fission was almost completed at the time of the operation.

E. *The Effect of Cutting Cells During Conjugation.*

During epidemics of conjugation pairs which had become firmly united were isolated and from these either the anterior or the posterior ends were removed (Fig. 10, *X* and *Y*). None of the pairs were cut through the center. The non-nucleated fragments always died shortly after the operation, while the larger ones either remained in contact for several hours or separated at once. Regeneration in these pieces is exceedingly slow and fission is greatly delayed. Truncated fragments (Fig. 11,

L and *R*) have been observed for days after the operation, swimming about rapidly but showing no sign of fission, or of regeneration of the lost end. Although the conjugating pairs were cut at the same level after separation one fragment was almost always smaller than the other (Fig. 11). The small one (*R*) generally dies, and the large one (*L*) divides to form a normal race, when both halves lived the smaller one finally divided and after many generations the descendants regained the normal size. The fission of all fragments of conjugating pairs was regular; I have never seen a large and a small cell formed by division in the original plane as so frequently happens when cells



10 11 12

FIG. 10. Conjugating cells showing region of the cut.

FIG. 11. The right and left halves of a conjugating pair after removal of the anterior end.

FIG. 12. Left and right halves of a conjugating pair grafted together.

are cut in the vegetative condition. This regularity would indicate that the division plane is lost during conjugation, and that when it is re-formed it is laid down in the center of the fragment.

In three of the operations the halves were pressed together so firmly by the edge of the knife that the fragments grew together at the cut surfaces. These grafted fragments (Fig. 12) showed great activity for a few days, but finally died without dividing. One of these was stained to see the nuclear condition. There was a large single nucleus in each half. If these grafts could be kept and fission induced, the regulation would, no doubt, be of value in throwing light on the problem of the "nucleus-protoplasm-relation."

F. *The Effect of Cutting Cells Shortly after Fission.*

In order to obtain cells at a definite period in their development they were removed from the stock culture, and as soon as

they had separated each cell was isolated in a drop of the cuture fluid. A period of two to five hours was allowed for growth, then the cells were cut and the further development of the nucleated fragments observed. The power of regeneration is present in these cells, but very few recover from the operation. In the actively growing cell the cytoplasm is in a less viscid state than it is in the vegetative cells, and it is for this reason that injury to the ectosarc results, in ninety per cent. of the operations, in the escape of the entire contents of the cell. In successful operations removal of the anterior or the posterior end give the same general results as in the vegetative cells. The most striking fact brought out is that as early as two and a half hours after fission the next division plane is determined. A brief description of a few experiments will demonstrate this.

R. 28. Two sister cells were isolated two and a half hours

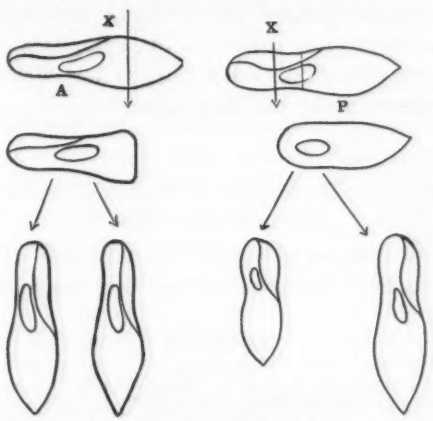


FIG. 13. Two sister cells cut at the same time, showing the formation of a normal race from the one from which the posterior end was removed, and the irregular cells formed from the cell from which the anterior end was removed.

after fission; the anterior end of one and the posterior end of the other were removed (Fig. 13, *A* and *P*). On the following day the nucleated fragments were active but had not yet divided. By noon of the same day *A* had formed two normal cells and *P* had divided into a small and a large cell. Later the smaller cells grew to the normal size; they were then killed.

R. 59. The anterior end of a cell was cut off four hours after fission. The nucleated fragment divided irregularly into a small anterior and a large posterior cell.

R. 30. One monster was obtained from a cell cut five hours after fission. Almost the entire anterior half was removed (Fig. 14, *X*). The wound closed in very slowly, the fragment increased in size (*C*), and not until the fourth day after the operation was any sign of division observed; then a constriction appeared near the anterior end which finally assumed the shape of an individual (Fig. 14, *D*). On the tenth day the mass divided into two irregular cells (*E* and *F*) each containing only one nucleus. It is evident that fission here was not only delayed but the whole mechanism of cell division was thrown out of order. The disturbance here was greater than when the cut was made further forward, and may have been due to injury to the nucleus.

In these young cells there was no sign of an increased rate of fission after removal of a part of the cytoplasm such as was observed in vegetative cells where three or four divisions followed in quick succession in the first twenty-four hours after the operation.

G. *A Comparison of the Behavior of the Nucleated Fragments from Four Different Races.*

In order to compare the power of regeneration in different races four distinct lines were selected and the following table compiled.

TABLE III.

Race.	Food.	Regeneration Followed by Normal Division.		Division in Original Plane.		No Regeneration.	
		Anterior End Removed. Per Cent.	Posterior End Removed. Per Cent.	Anterior End Removed. Per Cent.	Posterior End Removed. Per Cent.	Anterior End Removed. Per Cent.	Posterior End Removed. Per Cent.
C	M. Milk	67	100	33	9	0	0
M	M. Milk	23	25	59	30	18	45
E	Timothy	50	88	50	12	0	0
S	Timothy	25.5	43	49	30	25.5	27

Such a table as this demonstrates clearly that the power of regeneration varies greatly in different races. It also shows that in all four races there is greater disturbance when the anterior

end is removed than there is when the posterior end is cut off. This table does not show the tremendous differences in the behavior which result from the condition of individual cells at the time of the operation. There is a definite correlation between the behavior of fragments and the periods of depression to which cultures are subject. At such times a race which normally shows great power of regeneration fails both to regenerate and to divide. The power to regenerate is not so much a characteristic of a race as it is an indication of the vitality of the individual cell. *Paramecium* taken from a pure line will regenerate in ninety cases out of a hundred if the cytoplasm is in a viscid state and the animals are well fed. When the cells are starved or in a period of depression from other causes, the rate of division is slow and the power of regeneration is greatly reduced or altogether lost.

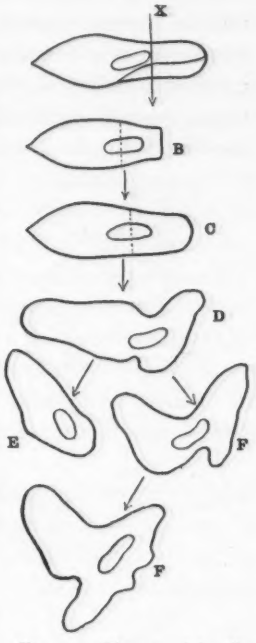


FIG. 14. Diagram giving the history of a fragment cut close to the nucleus.

H. *Can the Size of a Race be Reduced by Removal of Part of the Cytoplasm?*

In his extensive and careful investigations on *Paramecium* Jennings ('10) has shown that race size is inherited and that a new race of a different size does not arise within a pure line. Popoff ('09) claims that it is possible through experimental means to change the size of the race and to maintain the altered cell size for an indefinite period. The forms upon which Popoff made his observations were *Frontonia leucas* and *Stentor cæruleus*.

In order to determine whether or not a loss of cytoplasm alone would reduce the size of a race permanently I undertook a long

series of experiments on certain pure lines where the average size was definitely ascertained before the operation was performed, and the average length and breadth of all cells arising from small fragments was compared. If these measurements had been made from cells of the first few generations arising from such fragments the conclusion would have been reached that it is not only possible, but quite a simple matter to produce a small race from a large one. It is necessary to keep such cultures for many generations until after repeated divisions the normal size is regained. The method followed in these experiments may be illustrated by giving one example. All measurements were made with a micrometer under a Zeiss objective 3, ocular 1, after the cells were killed in Worcester's fluid.

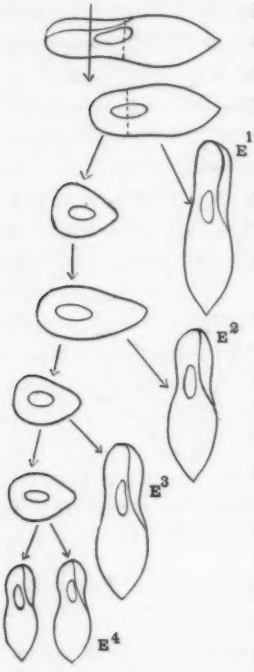


FIG. 15. Diagram showing the formation of normal races after irregular division

Experiment E.—The anterior end of the cell was removed (Fig. 15), and as soon as the nucleated fragment divided into a small and a large cell the two individuals were separated. The large piece continued to divide forming a normal race which we shall distinguish as E^1. The small piece after a period of growth divided irregularly into a small anterior and a larger posterior cell. The descendants of the posterior cell formed a race which is designated as E^2. The next division of the small piece was again irregular, the posterior cell exceeding the anterior in size. The race formed from this large cell was designated E^3. The small anterior piece at the next division formed two cells of equal size; these were called E^4. There were then four races E^1, E^2, E^3 and E^4 to be compared with the pure line E from which they arose. Careful measurements were made from time to time and during the course of the experiments, and all of the

races were kept under the same conditions as the control. The experiment was begun on March 14, and the first measurements made on the twenty-third showed the same average size in E, E^1 and E^2, but E^3 and E^4 were much smaller. The next measurements made on the twenty-sixth showed that E^3 had reached the average size, and a week later the small cells of E^4 were fully as large as the control race.

It is undoubtedly true that a large number of these small fragments, and most of the cells thrown off from the monsters, show signs of extreme feebleness and often die after one or two divisions. It is only those that formed races that are taken into consideration. Small fragments if possessing enough vitality to continue the race will sooner or later become as large as the parent stock. At the present time I have a race descended from a small anterior fragment formed by irregular division. The average size of these cells is slightly larger than that of the pure line from which they arose.

In this connection it is also interesting to note that after removal of the anterior ends of conjugating cells (Fig. 10, X) when for some reason the fragments differ in size (Fig. 11, L and R) the descendants of both halves finally regain the same average size.

General.

In considering the phenomena of regeneration and regulation in a unicellular organism one must take into account the fact that we have, in such a form as *Paramecium*, a highly differentiated structure. This differentiation is greater in the anterior end of the cell and diminishes posteriorly. Therefore it is not surprising that less disturbance results from removal of the posterior than of the anterior end. Regeneration follows the removal of the posterior end (Fig. 1, Z) in most cases, but this does not mean that the entire end is replaced before fission occurs Such fragments divide before the full size is regained, therefore regeneration amounts to nothing more than the healing over of the wound and the formation of new cilia; the regaining of the original body form is brought about by a gradual process of regulation through growth in volume. After the wound is healed

a new plane of division is laid down, and through fission two individuals of equal size are produced; when the new plane is not formed division takes place in the original plane and two cells of unlike size are produced. What causes this difference? Why does a fragment usually divide into two cells of equal size when the posterior end is cut off and of unequal size when the anterior end is removed? I have suggested that this is due to the position of the nucleus. When the cut is made close to the anterior end of the nucleus it is stimulated to divide at once while there is no such stimulation when the cut is made through the end of the body at some distance from the posterior end of the nucleus. Popoff claims that the volume of the cytoplasm can be regulated so that it accommodates itself to the volume of the nucleus. In this instance, however, it does not seem to be a question of balance between nuclear volume and cell volume. After removal of one end the fragment contains a whole nucleus and only two thirds to three fourths the normal volume of cytoplasm. After the first irregular division the small piece contains one half of the original nucleus and from one fourth to one third of the cytoplasm. Before the balance is established the cell may divide again into a small and a larger cell, or into two cells of the same size. When cut in half in the vegetative state the surviving fragment contains all of the nucleus and one half of the cytoplasm, yet these cell fragments divide in the normal manner.

It appears then that the ratio of the volume of the cytoplasm to the volume of the nucleus does not explain these results, and we must, therefore, look to some other source for light on this subject. If, as it appears, the division planes are laid down shortly after fission is it not possible that irregularities might result from the disturbance of these planes? I do not believe that there is any visible plane present, but there is strong indication of some such differentiation. If we consider a mature *Paramecium* as possessing a plane through the center as indicated by the double row of dots in Fig. 16 and two potential planes in the middle of each half, at the single row of dots, there are four normal individuals present in one cell. A cut made

through the center should be followed by normal division of the surviving half, and this is the case. If the cut is made through either of the other planes we should expect division in the original plane resulting in a small and a large individual, but if the cut were made in front of the most anterior plane or back of the most posterior one there would be two opposing division areas in the same fragment and a reorganization would be necessary; thus both planes would disappear and a new one would form which would bring about symmetrical division. The results of the experiments that I have made seem to support this theory, but in order to test the validity of the suggestion it would be necessary to make careful measurements of each cell used, and it would scarcely be possible to determine with exactness whether or not the cut were made through the region of a division plane or on one side of it.

FIG. 16. Diagram of mature cell showing regions of division planes.

The fact that a loss of cytoplasm from such a highly differentiated organism as *Paramecium* does not affect the size of the race is one more proof of the necessity of seeking some way of reaching the nucleus, for if some of the nuclear material could be removed the result might be very different. So far I have found it impossible to keep a fragment alive after injury to the nucleus. It is possible that after repeated removal of cytoplasm from the same cell a small race size could be established, but the difficulties of the operation and the great disturbance produced by it in the cell would render the experiment practically impossible.

SUMMARY.

1. When the anterior end of the cell was removed by a transverse cut through the peristome just in front of the macronucleus, thirty-four per cent. of the fragments regenerated the lost end and divided normally. When the posterior end was removed by a similar cut, back of the mouth, sixty-two per cent. of the nucleated fragments regenerated the lost end and formed a normal race.

2. Removal of the anterior end resulted in irregular division

in fifty per cent. of the nucleated fragments, while removal of the posterior end caused irregular division in only twenty per cent. of the pieces.

3. *Paramecia* cut in half transversely usually die as the nucleus is forced out by the pressure of the knife. When the nucleus remains in one of the halves regeneration is followed in that half by normal division.

4. Monsters develop from fragments composed of more than half of the protoplasm of a normal *Paramecium*, but never develop when a cell is cut exactly in half.

5. A fixed division plane is present in the normal cell as early as two and a half hours after fission. It is possible in vegetative cells, where fission has been delayed for a period, that more than one plane is laid down. This would account for the fact that after cutting off one end of the cell several divisions follow in quick succession.

6. The power of regeneration varies in different races, and in different individuals of the same race. It is the index of the condition of the cytoplasm.

7. The removal of a portion of the cytoplasm does not result in the production of smaller individuals. After several generations have been produced the normal size is regained.

Bryn Mawr, Pa.,
May 11, 1912.

LITERATURE.

Calkins, G. N.
'11 Effects Produced by Cutting *Paramecium* Cells. Biol. Bull., Vol. 1, p. 36.
Jennings, H. S., and **Hargitt, G. T.**
'10 Characteristics of the Diverse Races of *Paramecium*. Jour. Morph., Vol. 21, No. 4.
Lewin, K. R.
'10 Nuclear Relations of *Paramecium caudatum* during the Asexual Period. (Preliminary communication.) Vol. 16, Part 1.
Popoff, M.
'09 Experimentelle Zellstudien. 2. Ueber die Zellgrösse, ihre Fixierung und Vererbung. Arch. f. Zellforschung, 3, p. 124.

ON THE RECOGNITION OF SEX THROUGH EXTERNAL CHARACTERS IN THE YOUNG RAT.

C. M. JACKSON,

UNIVERSITY OF MISSOURI.

The rat is an animal widely used for scientific investigation. Especially through the work on growth by Donaldson and his associates, it has in some respects been more carefully studied than any other animal. The recognition of sex from external characters in the young living rats is therefore sometimes a matter of considerable importance in selecting animals for various lines of investigation.

That considerable inconvenience has been occasioned by difficulty in the early recognition of sex in the living rat is evident. King,[1] for example, states (p. 385) that "The sex of a newborn rat cannot be ascertained with any degree of certainty unless the animal is killed and dissected. When the rats are 14–16 days old, however, the sexes are easily distinguished as Dr. Stotsenberg has discovered, since the mammæ are clearly visible at this time. After this period the hair covers the entire body and it becomes very difficult to distinguish the sexes in the living young until they are several weeks old." Slonaker[2] similarly notes (p. 4) that "Owing to the fact that it is difficult to determine the sex of the young rats with accuracy the sexes were not distributed as I would have wished."

Since no method of distinguishing the sexes in the young rat (except that noted by King) has, so far as I know, been published, the following observations, which were made during a study of growth in the white rat, may be of some interest and value. While the observations were made exclusively upon the white or albino rat (*Mus norvegicus albinus*), they will doubtless apply

[1] Helen Dean King, "The Effects of Semi-Spaying and of Semi-Castration on the Sex Ratio of the Albino Rat (*Mus Norvegicus albinus*)." *Journal of Experimental Zoölogy*, Vol. 10, No. 4, 1911.

[2] James Rollin Slonaker, "The Effect of a Strictly Vegetable Diet on the Spontaneous Activity, the Rate of Growth, and the Longevity of the Albino Rat." Leland Stanford Junior University Publications. University Series, April 2, 1912.

also to the ordinary gray or brown rat (*Mus norvegicus*). A few observations upon young gray mice indicate that (at least with respect to the ano-genital distance and the size of the genital papilla) the same method may be utilized in distinguishing the sexes in this and perhaps other members of the genus.

TABLE I.

ANO-GENITAL DISTANCE IN YOUNG ALBINO RATS OF VARIOUS AGES.

Age.	Number of Each Sex.		Average Gross Body Weight (and Range).		Average Ano-genital Distance (and Range).	
	Male.	Female.	Male, Grams.	Female, Grams.	Male, Mm.	Female, Mm.
Newborn.	10	12	5.7 (5.1–6.3)	5.4 (4.7–6.3)	2.8 (2.5–3.0)	1.2 (1.0–1.5)
7 days . .	17	26	11.0 (8.8–12.9)	10.4 (6.7–12.2)	5.2 (4.5–6.0)	2.7 (2.0–3.5)
14 days . .	13	15	19.5 (17.0–21.9)	18.2 (15.3–19.9)	8.2 (7.0–9.0)	4.9 (4.0–6.0)
20 days . .	19	26	27.4 (15.6–40.0)	27.4 (19.7–41.6)	12 (10–14)	7 (6–9)
6–7 weeks	19	13	73.3 (57–114)	71 (23.5–95)	21 (17–25)	13 (9–15)

The observations may be grouped under four headings. The first and most important character to be noted is the *ano-genital distance*, which is the distance measured from the anal aperture to the base of the genital papilla (clitoris or penis). The measurements are summarized in Table I. They were made on the living animals at the various ages from birth to 6 or 7 weeks, when the sexes are easily distinguished by the ordinary characters. It will be observed that *in all cases the ano-genital distance in the male is much greater than in the female of the same age*. It averages nearly twice as great. Although, owing to the great range of variation in the size of the body, the largest distance recorded in the table for the female sometimes approaches the smallest found in the male for the same age, there is never *in any given litter* the slightest difficulty by this measurement in determining the sex with ease and accuracy.

A second characteristic is found in the *size of the genital papilla*. This (anlage of penis or clitoris) is always larger and more prominent in the male than in the female. This difference is clearly evident when the sexes are compared in individuals of the same litter.

A third characteristic is the *presence of the mammæ*, already mentioned by King as observed by Stotsenberg. I have con-

firmed the statement that the mammæ (nipples) are readily visible in the female of 14–15 days, and that after that time they become hidden by the hair. During lactation, of course, they again appear. I have also observed further that the nipples are visible at an earlier age than that mentioned. During the entire second week, and at least in some cases during the first week, they can be noted by careful observation in the female. From the time they first appear, the definite number (six pairs) is present.

When the hair coat becomes well developed, at about 16 or 17 days of age (when also the eyes are opened), a fourth sexual characteristic may be noted. In the male, a small area just ventral to the anus remains bare. In later stages it becomes to a certain extent covered with short hairs, but always remains relatively bare. This corresponds to the dorsal part of the scrotal area, as may be noted after the sixth week, when the testes occasionally descend. In the female, there is a corresponding bare, or relatively bare, strip which extends all the way from the anus to the genital papilla (clitoris). The part of this bare strip just dorsal to the clitoris corresponds to the vaginal aperture; but the aperture does not appear until the middle or end of the second month. In one case, a female bore a litter at the age of 10 weeks, and must therefore have become pregnant at about 7 weeks. Lantz[1] cites a case where a white rat is said to have given birth to 11 young when only 8 weeks old, and which therefore must have bred when only 5 weeks old.

It is evident from the foregoing that of the four characteristics noted for distinguishing the sexes in young rats, one (visibility of nipples) applies only before the age of about 16 days; one (bare areas in ano-genital region) applies only after that age; while the other two (ano-genital distance and size of genital papilla) apply to all ages.

[1] David E. Lantz, Natural History of the Rat. In "The Rat and its Relation to the Public Health," by various authors. P. H. and M. H. Service, Washington, 1910.

THE LINKAGE OF TWO FACTORS IN DROSOPHILA THAT ARE NOT SEX-LINKED.

T. H. MORGAN AND CLARA J. LYNCH.

In addition to the eight sex-linked factors described for *Drosophila*, a number of other factors have been found that are not sex-linked. They are now being studied in order to discover which, if any, of them are linked to each other. Two that have been found to be linked are here described. These are the yellow

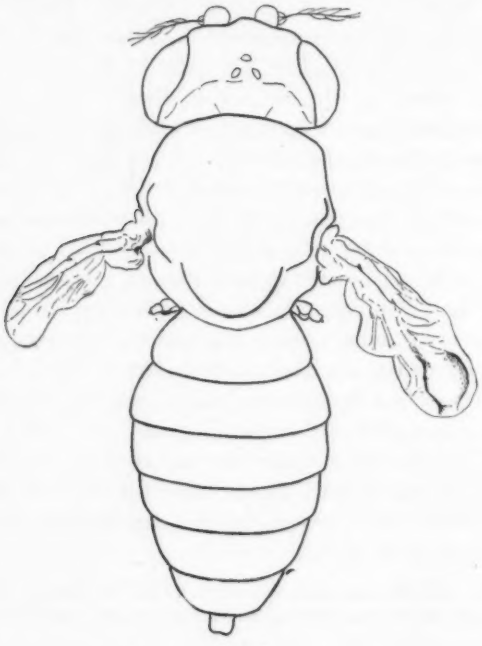


FIG. A.

factor in the absence of which the flies are black, and a factor (in the complex of factors that produces the normal wing) in the absence of which the fly is wingless.

In previous papers on *Drosophila* a large amount of data has been given showing that in regard to any sex-linked factor, the, yellow factor, *Y*, follows Mendel's law of independent segregation; in other words, the yellow factor segregates independently of all

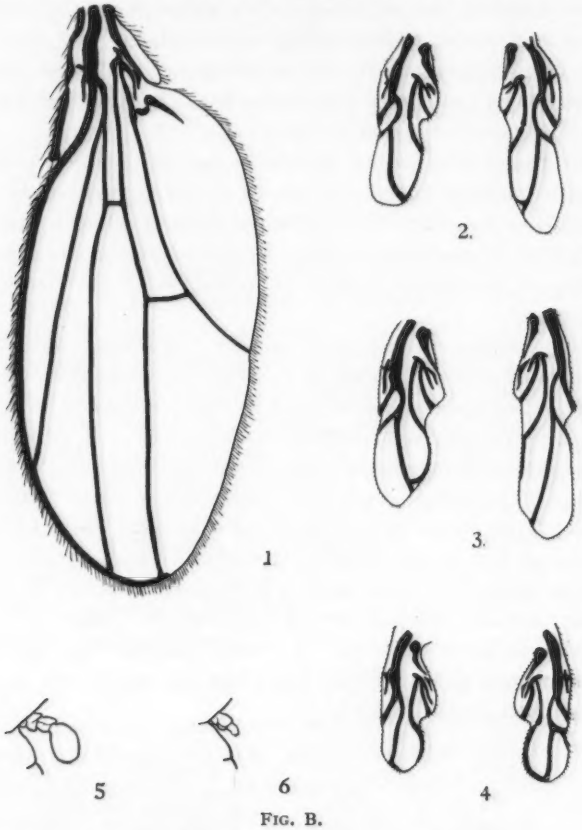


FIG. B.

sex-linked factors. The fact that wingless flies do not show sex-linked inheritance has also been reported, but the character of the wing itself and its mode of origin have not been fully described.

The size in relation to the body, and general character of the wings are shown in Fig. A. They are little more than scales held

out at right angles at the sides of the body. The details of the venation are shown in the three pairs of wings shown in Figs. B, 2, 3 and 4, while for comparison a normal ("long") wing drawn to scale is shown in Fig. 1.

The wingless wing represents in the main the basal portion of the normal wing. The marginal vein is missing, but the cross vein connecting the third and fourth longitudinal veins is often present as an apparently marginal vein. The basal parts of all five longitudinal veins are present.

The balancers are much reduced in size and modified in character. A normal balancer is shown in Fig. 5 and one from a wingless fly in Fig. 6. The terminal segment which is large in the wild fly is represented by only a trace and the second segment is reduced, but the basal segment approaches in size that of the wild type.

The wingless flies appeared in the "truncate" stock and represented at first the wingless condition of the truncate type of wing; owing to the low viability of the truncate flies the winglessness was transferred to normal stock, but as it is difficult or impossible to distinguish between Wingless of truncate stock and wingless of long-winged stock the material was not at first quite homogeneous except in so far as being wingless. There was also present at first in the wingless stock another character, namely, balloon wings, which appeared at about the same time as Wingless in the truncate stock and proved as difficult to separate from the Wingless as Truncate itself. As a result, balloon wings appear in the first cross given below. The cross was made over a year ago by Miss E. M. Wallace.

P_1		F_1		F_2	
Wingless ♂	=	Winged ♂	=	Winged ♀,	1,136
Winged ♀		Winged ♀		Winged ♂,	1,038
				Wingless ♀,	259
				Wingless ♂,	236
				Balloon ♀,	12
				Balloon ♂,	3

The sum of the winged and balloon is 2,189 and that of the wingless is 495, which gives a ratio of 4.42 to 1. It is clear that

winglessness is recessive to normal, that it is not sex-linked, and that it has a lower viability than has the normal. The reciprocal cross gave:

P_1		F_1		F_2	
Winged ♂	=	Winged ♀	=	Winged ♀,	694
Wingless ♀		Winged ♂		Winged ♂,	639
				Wingless ♀,	146
				Wingless ♂,	127
				Balloon ♀,	4
				Balloon ♂,	1
				Miniature ♀,	1
				Miniature ♂,	24

One at least of the grandparental wingless flies must have been responsible for the miniatures, but the numbers are too small to affect the other ratio seriously. There were 1,363 long (including balloon and miniature) flies, and 273 wingless, or 4.9 to 1.

In a recent cross between long-winged ♀ and wingless ♂ there were present in the F_2 generation 1,081 long winged F_2 flies, and 280 Wingless; a ratio of 3.9 to 1. The reciprocal cross gave in F_2 1,213 long-winged flies and 273 Wingless or a ratio of 4.4 to 1. Compared with the preceding the results show that the viability of the Wingless has not changed. It is curious to find that the F_2 ratio in this cross and its reciprocal differ in both instances in the same direction as in the earlier cross, but this may be only a coincidence.

Linkage of the Yellow Factor, *Y*, and the Wing Factor, *W*.

The linkage in question is most simply shown in the following result: wingless Grays were mated to long-winged Blacks, and gave in F_1 long-winged gray males and females. These inbred gave in the F_2 generation:

Winged Gray.		Winged Black.		Wingless Gray.		Wingless Black.	
♂	♀	♂	♀	♂	♀	♂	♀
854	1,004	410	506	226	329	0	0

There are no wingless black flies in the F_2 generation, which the Mendelian expectation calls for. Their absence can only be

explained by strong linkage of the yellow factor and the factor for wings. The Mendelian expectation (unmodified by linkage and viability) is three long Grays to one long Black (3 : 1). *There are actually 1,858 long gray flies to 916 long black, or a ratio of 2 to 1. This is the linkage ratio when two strongly or completely linked factors are concerned.*

Since the males and females count equally in the result it is not necessary to separate them. We are indebted to Mr. A. H. Sturtevant for the following F_2 count derived from the same F_1 material as the last. We are also indebted to him for being first in pointing out the interpretation of this result.

Winged Gray.	Winged Black.	Wingless Gray.	Wingless Black.
458	230	185	0

There are 458 winged Grays to 230 winged Blacks, or almost exactly 2 : 1.

In the following analysis Y = yellow factor; y its absence; B = black factor (sex-linked), and b its absence. The formula for the female gray fly is $YBXYBX$, and for the male, $YBXYb$. The formula for the female black fly is $yBXyBX$, and for the male is $yBXyb$.

The evidence indicates that the wingless condition differs from the winged condition by a loss. Large W may then stand for that " factor " in the original complex that has been lost. In this sense large W means in this case Wings (here long); *and small w stands for this loss and in the formulæ represents the wingless condition, which is of course the result of the interaction of all the remaining wing factors.*

	wYBX—wYBX	Wingless gray ♀.
	WyBX—Wyb	Winged black ♂.
F_1	*wYBX WyBX*	Winged gray ♀.
	wYBX Wyb	Winged gray ♂.
Gametes of	*wYBX—WyBX*	Eggs.
F_1	*WyBX—wYBX—Wyb—wYb*	Sperm.
	wYBX WyBX	Winged gray ♀.
	wYBX wYBX	Wingless gray ♀.
	WyBX WyBX	Winged black ♀.
	WyBX wYBX	Winged gray ♀.

wYBX Wyb	Winged gray ♂.
wYBX wYb	Wingless gray ♂.
WyBX Wyb	Winged black ♂.
WyBX wYb	Winged gray ♂.

In the F_1 female, gametes having the composition *WYBX* and *wyBX* would be expected on any assumption except that of strong linkage of *wY* and *Wy*, and likewise in the male, the gametes *WYBX*, *wyBX*, *WYb* and *wyb* would be expected. For the sake of convenience I have omitted to represent the cross-over gametes in the above analysis.

In another cross, miniature-winged, black, white-eyed flies were mated to wingless, gray, red-eyed flies. The result is complex, but only in the sense of giving in one cross 20, F_2, classes, and in the reciprocal cross 13, F_2, classes. The results, however, as far as linkage is concerned, are perfectly evident. The fact is also brought out that the factor for Wings shows no linkage to the sex-linked factor (*C*) for eye color, and to (*R*) the factor in the absence of which the wings are miniature.

Miniature White Bl ♂ by Wingless Red Gray ♀

GR Long ♂ and ♀

F_2 Females

GRL	*BlRL*	*GR* Wingless
532	268	235

Males

GRL	*GWhL*	*BlRL*	*BlWhL*	*GRM*	*GWhM*	*BlRM*	*BlWhM*	*GRW-g-s*	*GWhW-g-s*
173	49	81	38	92	165	30	52	117	90

There are three classes of females and ten classes of males. The wingless flies only occur as Gray—no black wingless flies being present. The alteration in the ratios, due to the linkage between the two sex-linked factors *C* and *R*, is neglected here. The analysis follows:

	wRMCBYX—wRMCBYX	Wingless red gray ♀.
	WrMcByX—Wrmcby	Min. white black ♂.
F_1	*wRMCBYXWrMcByX*	Winged gray red ♀.
	wRMCBYXWrmcby	Winged gray red ♂.

Gametes of F_1 — *WRMCByX–wRMCBYX–WrMcByX–wrMcBYX* } eggs
WRMcByX–wRMcBYX–WrMCByX–wrMCBYX }
WRMCByX—wRMCBYX—Wrmcby—wrmcbY Sperm.

Females.

WRMCByXWRMCByX	Long red black.
WRMCByXwRMCBYX	Long red gray.
WRMcByXWRMCByX	Long red black.
WRMcByXwRMCBYX	Long red gray.
wRMCBYXWRMCByX	Long red gray.
wRMCBYXwRMCBYX	Wingless red gray.
wRMcBYXWRMCByX	Long red gray.
wRMcBYXwRMCBYX	Wingless red gray.
WrMcByXWRMCByX	Long red black.
WrMcByXwRMCBYX	Long red gray.
WrMCByXWRMCByX	Long red black.
WrMCByXwRMCBYX	Long red gray.
wrMcBYXWRMCByX	Long red gray.
wrMcBYXwRMCBYX	Wingless red gray.
wrMCBYXWRMCByX	Long red gray.
wrMCBYXwRMCBYX	Wingless red gray.

Males.

WRMCByXWrmcby	Long red black.
WRMCByXwrmcbY	Long red gray.
WRMcByXWrmcby	Long white black.
WRMcByXwrmcbY	Long white gray.
wRMCBYXWrmcby	Long red gray.
wRMCBYXwrmcbY	Wingless red gray.
wRMcBYXWrmcby	Long white gray.
wRMcBYXwrmcbY	Wingless white gray.
WrMcByXWrmcby	Min. white black.
WrMcByXwrmcbY	Min. white gray.
WrMCByXWrmcby	Min. red black.
WrMCByXwrmcbY	Min. red gray.
wrMcBYXWrmcby	Min. white gray.
wrMcBYXwrmcbY	Wingless white gray.
wrMCBYXWrmcby	Min. red gray.
wrMCBYXwrmcbY	Wingless red gray.

The reciprocal cross gave the following results.

Minature White Bl ♀ by Wingless Red Gray ♂

Gray Red ♀ and Miniature Gray White ♂

Females.

GRL	*GWhL*	*BlRL*	*BlWhL*	*GRM*	*GWhM*	*BlRM*	*BlWhM*	*GRW-g-s*	*GWhW-g-s*
220	45	88	45	96	197	46	62	113	95
468				401				208	

Males.

GRL	*GWhL*	*BlRL*	*BlWhL*	*GRM*	*GWhM*	*BlRM*	*BWhM*	*GRW-g-s*	*GWhW-g-s*
225	96	120	35	92	201	41	99	131	105
473				433				236	

In this reciprocal cross there are ten classes each of females and males. Each wingless class is made up (as in the other cross also) of wingless long and wingless miniature. These, if they could be separated, would give, in all, twelve classes of males and females.

The analysis of this cross is similar in principle to that given above and can readily be reproduced by the method used for that cross. How closely the yellow factor and that for Wing are united will be determined later by suitable tests.

On the assumption that factors are contained in chromosomes, it will be apparent that this new case of linkage means that both *W* and *Y* are contained in a chromosome different from the sex chromosome.

After the foregoing results were completed, a doubt arose as to whether we could with certainty detect black wingless flies, even if present, since they would lack one of the most characteristic features of black flies, namely, the wings. We determined to test some of the F_2 flies by mating to long-winged black flies, for we thought, at that time, that this would be a legitimate test for the presence of black flies amongst the wingless offspring. But while it is true that the appearance of black offspring would be expected, if black flies were present, nevertheless the appearance of such flies does not in itself show that black flies were actually present amongst the flies tested; for, as the following analysis will show, crossing over may have occurred when the gametes were produced, and yet no F_2 black

wingless flies result. The test showed, in fact, that crossing over had occurred, but the result was obscured, because, necessarily, the F_2 flies would still be gray, unless the crossing over had occurred simultaneously in both sexes and the cross-over gametes happened to meet. The following experiments will illustrate this point:

Seven F_2 wingless males were paired with 15 long-winged black females, and gave 133 gray females, and 96 gray males.

But five wingless females mated to fifteen long black males gave: Gray, ♀, 64; ♂, 61; Black, ♀, 8; ♂, 7. The last result shows that crossing over had occurred in the gametes of one F_1 fly, which gave rise to one fly (at least) that was heterozygous for color, and from this fly came the black flies that appeared in the test. The point of particular interest is that this crossing over had not been apparent in the F_2 gray flies, and the reason for this is not far to seek. If crossing over between *wY* and *Wy* in the gametes of the F_1 generation had occurred, the gametes would be: *WYB*, *wYB*, *WyB*, *wyB*.

If this fly were fertilized by an ordinary F_1 male, whose gametes would be *wYB* and *WyB*, all the wingless flies would be gray, and those grays derived from the crossing-over (however rare their occurrence) would be heterozygous for color. Only a back cross by black flies would make evident in the next generation that crossing-over had really occurred. How often it occurs remains to be discovered by further experiments. The ratio of 2 to 1 in the F_2 gray flies shows, however, that crossing-over is not common, and the absence of black wingless flies in the F_2 generation indicates this even better. For, if crossing over were common the cross-over gametes of the F_1 flies would occur often enough for like to meet like and produce black wingless flies.

ON COUPLING OF CERTAIN SEX-LINKED CHARACTERS IN DROSOPHILA.

JOHN S. DEXTER.

In the course of his experiments on the modes of inheritance in *Drosophila*, Morgan has called attention[1] to the fact that when flies bearing two or more pairs of characters of certain sorts are crossed, these characters appear in the F_2 generation coupled as in the original combination (*i. e.*, in the grandparents). This coupling is not complete, but is much larger than is to be expected if the different pairs of characters were independently mendelizing. In a letter to me last autumn he suggested that "in order to find out if there is here some deep-lying principle or only some irregularity, it will be necessary to obtain very large numbers of the F_2's; let us say ten or twenty thousand."

Morgan has offered an explanation of this coupling[2] on the supposition that the factors concerned in the production of the characters lie near together in the chromosomes, and that the twisting of homologous chromosomes about each other in the strepsinema stage of gametogenesis which causes both maternal and paternal characters to lie on each side of the double chromosome, does not separate certain factors that lie very close together, so that when the chromosomes split, these factors will both occupy the same gamete.

It was in order to test these theories that I undertook, at Dr. Morgan's suggestion, to raise "large numbers of the F_2's."

Dr. Morgan's kindly advice and aid have helped me a great deal in this work. I wish also to record here and express my appreciation of the enthusiastic assistance of Miss Margueritte Harmon and Mr. Felix Gustafson, without whose aid the work could not have been done at this time.

I began with pure stock of *Drosophila* of two kinds: Flies with normal body color and red eyes (this is the normal wild fly),

[1] *Jour. Exp. Zoöl.*, Vol. 11, No. 4, Nov., 1911, p. 393.

[2] *Science*, N.S., Vol. 34, No. 873, Sept., 1911, p. 384.

and flies with a yellow body and white eyes; both stocks were sent to me by Morgan. The origin of these mutations, and the facts of their individual inheritance, have been described by Morgan[1] in earlier papers.

In speaking of the flies from this time on, I shall use the letter *N* to signify the presence of the factor for normal body color, *Y* for yellow body color, *R* for red eye, and *W* for white eye. Of these factors, *N* is dominant over *Y*, and *R* over *W*. The factors *N* and *R* are only found in association with the *X*-chromosomes, which is duplex in the female but simplex in the male. This means, of course, that *N* and *R*, as used here, are sex-linked characters. It is not necessary to assume that any of these factors are units but that they act as units in the following experiments.

According to the theory to be tested we have the following data as to the gametic constitution of the flies. A pure *NR* female has the constitution *NRX—NRX*, and all her gametes bear the factors, *NRX*. A pure *NR* male has the constitution *NRX—YW*, and the sperms bear either of two combinations of factors, *NRX* or *YW*. A pure *YW* female has the constitution *YWX—YWX*; the gametes all bear *YWX*. The pure *YW* male has the constitution *YWX—YW*, and his gametes bear either *YWX* or *YW*.

Experiment I.

In experiment I, a pure *NR* female was crossed to a pure *YW* male. The results to be expected in the F_1 and F_2 generations if eye color and body color are independently mendelizing, are shown in Table I.

Table I.

P_1.	*NRX—NRX* = *NR*♀	*YWX—YW* = *YW*♂
	Gametes *NRX*	*YWX*, *YW*
F_1.	*NRX—YWX* = *NR*♀	*NRX—YW* = *NR*♂
	Gametes (1) *NRX*, (2) *YWX*, (3) *YRX*, (4) *NWX*	*NRX*, *YW*
F_2.	*NRX—NRX* = *NR*♀	*NRX—YW* = *NR*♂
	YWX—NRX = *NR*♀	*YWX—YW* = *YW*♂
	YRX—NRX = *NR*♀	*YRX—YW* = *YR*♂
	NWX—NRX = *NR*♀	*NWX—YW* = *NW*♂

[1] *Science*, July 22, 1910, p. 120; *Science*, March 31, 1911, p. 496; *Jour. Exper. Zoöl.*, November, 1911, p. 365.

This table shows that all members of the F_1 generation should have the appearance of normal *NR*'s. In my experiments, this was true without exception.

The females of the F_1 generation should produce four classes of eggs, representing all combinations of *N*, *R*, *Y* and *W*. The males should produce sperms of two classes. The combination of these gametes should produce in the F_2 generation equal numbers of males and females, but all of the females should be *NR*, while one fourth of the males should belong to each of the following classes: *NR*, *YW*, *YR*, *NW*.

If, on the contrary, the *N* and *R* are completely coupled we have the same result in the F_1 generation, but the gametes of the F_1 females would be of two classes only, viz., *NRX* and *YWX*, while those represented in the table by (3) and (4) would be absent. Therefore there would occur in the F_2 generation *NR* females as before, and only two kinds of males, one half being *NR* and the other half *YW*.

Table II. shows the actual results of this experiment. From 24 bottles I raised 11,394 flies. Of these 6,081 were females. All but one of these females were *NR*. The other was *NR* on the left side and *YW* on the right, and may be for present purposes ignored[1] as an anomaly. Of the males there were of class *NR*, 2,870; class *YR*, 34; class *NW*, 36; class *YW*, 2,373. Whatever the explanation may be, it is obvious that coupling has occurred. It is equally obvious that the coupling is not complete.

The result for the individual bottles accorded with that of the total. There is in every bottle except number 6, a larger number of *NR* males than of *YW* males. This is without doubt due to a greater vitality in the normal flies than in the mutants, for also in the pure cultures the normal flies are much more fertile than the others. This matter is discussed by Morgan in one of the papers mentioned[2] above, under the heading, "The Fertility of Deficient Mutations." I have no reason to dissent from the statements made there. The reasons for this infer-

[1] This fly was active and except for color appeared to be normal. I attempted to mate her to *YW* males, but she appeared to be sterile, and after two weeks she was preserved for histological study.

[2] *Jour. Exper. Zoöl.*, Nov., 1911.

tility are now being investigated. At present, at least, the fact is very apparent, not only in my own results, but also in those published by Morgan.

TABLE II.

TABLE SHOWING RESULTS OF EXPERIMENT I.

Bottle No.	*NR*♀.	*NR*♂.	*YR*♂.	*NW*♂.	*YW*♂.	Total.	
1	201	92	4	3	81	381	
2	161	89	1	1	77	329	
3	282	123	1	1	90	497	
4	351	155	0	3	127	636	
5	46	28	1	1	18	94	
6	96	37	0	0	40	173	
9	108	46	1	0	33	188	
10	267	147	5	0	126	545	
13	253	144	0	1	84	482	
14	183	104	1	1	72	361	
15	253	144	1	1	135	534	
17	317	143	1	2	123	586	
19	352	165	1	2	132	652	
20	292	121	3	3	104	523	
21	277	146	2	1	102	528	
22	280	114	0	0	99	493	
23	136	63	0	0	72	271	
24	160	80	0	1	59	300	
25	127	73	2	0	55	257	
26	242	118	0	0	64	424	
27	544	234	3	2	233	1,016	1 abnormal ♀
50	503	203	5	8	153	872	$\frac{1}{2}NR$, $\frac{1}{2}YW$
51	325	154	0	3	148	630	
52	324	147	2	2	146	621	
Totals	6,080	2,870	34	36	2,373	11,393	11,394

EXPERIMENT II.

In this experiment, the reverse cross was made, viz., pure *YW* females by pure *NR* males. According to the theory if no coupling occurred, the F_1 and F_2 generations should give the results shown by Table III.

TABLE III.

P_1.	*YWX*—*YWX* = *YW*♀	*NRX*—*YW* = *NR*♂
	Gametes *YWX*	*NRX*, *YW*
F_1.	*NRX*—*YWX* = *NR*♀	*YWX*—*YW* = *YW*♂
	Gametes (1) *NRX*, (2) *YWX*, (3) *YRX*, (4) *NWX*	*YWX*, *YW*
F_2.	*NRX*—*YWX* = *NR*♀	*NRX*—*YW* = *NR*♂
	YWX—*YWX* = *YW*♀	*YWX*—*YW* = *YW*♂
	YRX—*YWX* = *YR*♀	*YRX*—*YW* = *YR*♂
	NWX—*YWX* = *NW*♀	*NWX*—*YW* = *NW*♂

The table shows that in the F_1 generation there should be only *NR* females and *YW* males. My studies completely bore out this conclusion.

As in the previous experiment, the females of the F_1 generation should produce four classes of eggs, representing the various combinations of the factors concerned. The males should produce two classes of sperms. The union of these gametes should produce in the F_2 generation equal numbers of males and females of the four classes, *NR*, *YW*, *YR*, *NW*.

Complete coupling would give only two classes of eggs and hence only two classes of males and females, viz., *NR* and *YW*. Table IV. shows the results actually obtained. From 24 bottles there hatched 9,626 flies, distributed as follows: *NR* females, 2,761; *NR* males, 2,462; *YR* females, 30; *YR* males, 29; *NW* females, 21; *NW* males, 24; *YW* females, 2,204; *YW* males, 2,095.

Here again the coupling occurs, and again the coupling is not complete. We find also the deficiency of males and of mutants

TABLE IV.

GIVING RESULTS OF EXPERIMENT II.

Bottle No.	*NR* ♀.	*NR* ♂.	*YR* ♀.	*YR* ♂.	*NW* ♀.	*NW* ♂.	*YW* ♀.	*YW* ♂.	Total.
7	101	74	0	0	0	4	102	76	357
8	46	48	2	0	0	0	40	36	172
11	63	63	0	0	3	0	64	68	261
12	87	80	1	2	1	0	67	75	313
16	74	88	1	0	0	3	79	80	325
29	82	81	0	1	1	1	86	77	329
31	206	168	1	5	0	1	176	161	718
32	126	111	0	2	1	3	122	120	485
33	139	130	5	1	0	2	112	137	526
34	153	152	1	1	2	1	116	101	537
35	76	42	0	0	0	3	19	31	171
36	78	55	0	1	0	1	56	51	242
37	75	74	1	0	0	0	56	64	270
39	48	39	0	2	0	0	30	24	143
40	46	45	0	0	0	0	41	43	175
54	114	103	4	4	2	0	96	81	404
55	119	100	1	1	1	1	78	74	375
56	178	156	2	1	3	1	138	153	632
57	137	118	1	2	2	0	118	104	482
58	183	181	3	3	1	0	152	130	653
59	184	143	0	0	1	0	139	100	567
60	202	190	5	2	1	0	160	156	716
61	195	153	1	1	1	3	121	124	599
62	49	58	1	0	1	0	36	29	174
Total	2,761	2,462	30	29	21	24	2,204	2,095	9,626

when compared with the normal females. The figures in either of these experiments show an average of about eighty-five individuals in which the coupling persists to one in which the factors are interchanged.

Experiment III.

After carrying the above experiments to near their conclusion I suspected that perhaps there was some reason unknown to me which made *N* naturally couple with *R*, no matter what the original combination might have been. That my suspicions were ill-founded was demonstrated by the following experiments.

In the first of these, I selected from the classes of *NW* females and *YR* males of the F_2 generation (Table III.) obtained in Experiment II., virgin individuals and mated them. The gametic constitution of these and the nature of their offspring according to the theory are shown in Table V.

Table V.

The P_1 Generation of this Table Consists of Members of the F_2 Generation of Experiment II.

P_1.	*NWX—YWX* = *NW*♀	*YRX—YW* = *YR*♂
Gametes	*NWX*, *YWX*	*YRX*, *YW*
F_1	(1) *NWX—YRX* = *NR*♀	(3) *NWX—YW* = *NW*♂
	(2) *YWX—YRX* = *YR*♀	(4) *YWX—YW* = *YW*♂

This table shows that equal numbers of *NR* females, *YR* females, *NW* males, and *YW* males should be produced. I obtained:

NR females, 45	*NW* males, 52
YR females, 50	*YW* males, 47

I now took some of the virgin females from the *NR* group and inbred them to the *YW* males. It should be recalled that the parents of the F_2 generation in Experiment II. were also of these classes, and therefore, as shown by Table VI., if body color and eye color were not to couple, we should expect the same classes of individuals in the F_2 generation in each case. If coupling occurred here (*i. e.*, if having "broken" once, should not give any tendency to do so again), we should expect here, not the same results as those obtained in the previous experiment, but rather that the classes that were then small should now be the large classes, while the large ones should be now small.

I started six bottles of this cross, and in every case the classes *YR* and *NW* were in the majority. But in five of the bottles the food was very wet and the hatch poor. None of them gave any *YW*'s at all. Also, in every case the class *NW* far outnumbered the class *YR*. In the sixth bottle (see Table VII., Bottle No. 44) the food was in better condition (*i. e.*, was drier), the hatch larger, the ratios nearer the expected, and class *YW* represented. This would seem to indicate some relation between the dark body color and the relative dampness of the food, though I realize that this suggestion requires further investigation.

TABLE VI.

The members of F_1 are taken from classes (1) and (4) of Table V.

F_1.	*NWX—YRX* = *NR*♀	*YWX—YW* = *YW*♂
Gametes	(1) *NRX*, (2) *YWX*, (3) *YRX*, (4) *NWX*	*YWX*, *YW*
F_2.	*NRX—YWX* = *NR*♀	*NRX—YW* = *NR*♂
	YWX—YWX = *YW*♀	*YWX—YW* = *YW*♂
	YRX—YWX = *YR*♀	*YRX—YW* = *YR*♂
	NWX—YWX = *NW*♀	*NWX—YW* = *NW*♂

TABLE VII.

GIVING RESULTS OF EXPERIMENT III.

Bottle No.	*NR*♀.	*NR*♂.	*YR*♀.	*YR*♂.	*NW*♀.	*NW*♂.	*YW*♀.	*YW*♂.	Total.
41	1	1	17	19	63	37	0	0	138
42	2	1	10	6	66	55	0	0	140
43	1	0	3	1	44	47	0	0	96
44	0	2	91	90	103	115	2	0	403
45	1	1	9	13	41	25	0	0	90
46	0	3	11	7	74	40	0	0	135
Total	5	8	141	136	391	319	2	0	1,002

That the coupling here is in accord with that of the previous experiments is clear. A study of bottle number 44 in which the food was not wet and sticky will present an even fairer view of the case, as well as one in which the results are nearer those expected.

EXPERIMENT IV.

Here again I selected from virgin members of the F_2 generation of Experiment II., this time crossing *YR* females and *NW* males.

Their supposed gametic constitution and the results to be expected in the F_1 generation are shown in Table VIII.

TABLE VIII.

The P_1 generation consists of members of the F_2 generation of Experiment II. (see Table III.).

P_1.	*YRX*—*YWX* = *YR*♀	*NWX*—*YW* = *NW*♂
Gametes	*YRX*, *YWX*	*NWX*, *YW*
F_1.	(1) *YRX*—*NWX* = *NR*♀	(3) *YRX*—*YW* = *YR*♂
	(2) *YWX*—*NWX* = *NW*♀	(4) *YWX*—*YW* = *YW*♂

The table shows that there should be produced in equal numbers *NR* females, *NW* females, *YR* males, and *YW* males. The actual hatch consisted of

NR females, 116	*YR* males, 82
NW females, 116	*YW* males, 100
	NW males, 2

I am quite unable to account for the occurrence of the two *NW* males, except through mutation. Rather than offer this rather wild guess, I shall not attempt to explain it at all. It will be observed, however, that it could not possibly have been that the *YR* females were not virgin when the experiment was begun, for no known mating will produce an *NW* male from such a female. Also, it would be an unbelievable coincidence that should produce on the same day—the fifth after the beginning of the hatch—two such males on account of contamination of the food.

As in experiment III., I mated the virgin *NR* females to the *YW* males, so that Table VI. accounts for the results in the F_2 generation in the same way that it did for experiment III. The discussion, also, of the probabilities for the F_2 generation in that experiment applies equally well to this experiment.

I started only one bottle of this kind (No. 47). From it were produced:

NR females, 2;	*YR* females, 81;	*NW* females, 89;	*YW* females, 1;
NR males, 1;	*YR* males, 82;	*NW* males, 90;	*YW* males, 0.

A comparison of these figures with those of bottle No. 44 of experiment III. will show a very close agreement of the one to the other.

Discussion of the Problem in the Light of the Experiments.

In the above experiments we find Morgan's observations verified.

1. A coupling occurs in the F_2 generation in which the coupled factors are those that were associated in the grandparents.

2. This coupling is by no means complete.

3. The females of any class as a rule outnumber the males.

4. The classes which lack the factor for red eye or normal body color contain a smaller number of individuals than those which possess this factor, and the class lacking both of them is the smallest of all.

Perhaps the last two conclusions may be stated: The vitality of any class of individuals is increased according to the number they possess of factors found in the normal (*NR*) female. On this assumption, the reason why the *NR* males are less numerous than the *NR* females would be accounted for by their having the simplex rather than the duplex gametic constitution. Perhaps an individual has an added vitality even through the possession of two rather than one of the factors for sex, as in the case of the *YW* females as opposed to the *YW* males. I do not desire to urge the case, particularly since I understand Morgan is conducting investigations that will throw more light on the subject.

The particular problem presented by these experiments is to explain why the coupling occurs but is not complete. This problem perhaps can be finally settled only by the cytologist.

Morgan's suggestion, based on the observations of Jannsens, on the twisting of the chromosomes, I understand as follows. The factors through the influence of which any particular character appears in an individual, are represented in the chromosomes by material particles which have always the same relative linear position. "When the parental pairs (in the heterozygote) conjugate, like regions will stand opposed. There is good evidence to support the view that during the strepsinema stage, homologous chromosomes twist around each other, but when the chromosomes separate (split), the split is in a single plane."[1]

[1] Morgan, in *Science*, Sept. 22, 1911, p. 384.

Now the supposition is that if two of the factors lie close together on the same side of the double chromosome, they may escape separation by the splitting. This means that the factors must be closer together than half the distance around one complete turn of the spirally twisted chromosomes. This length is the maximum possible in order to allow two factors to be coupled on account of proximity. This length might be indefinitely diminished so that many factors of one of the parental chromosomes might lie closely approximated on the same side of the split, and therefore be coupled.

In order to make the situation clearer to myself, I took two pieces of one fourth inch rubber tubing, eighteen inches long, and marked off, with ink, lengths of one inch. Each tube represented one chromosome of a homologous pair, and the inch lengths represented the factors. I marked the factors on one chromosome with odd numbers, 1, 3, 5, 7, etc., to 35. This chromosome I called maternal. The factors of the other I marked with 2, 4, 6, 8, etc., to 36, and called this chromosome

TABLE IX.

The two vertical columns named *r* (right) and *l* (left) contain all the factors of both parents. Each column represents one gamete and contains that half of the factors not found in its fellow. Odd numbers represent maternal, even numbers paternal characters.

r	*l*	*r*	*l*	*r*	*l*	*r*	*l*	*r*	*l*	*r*	*l*	*r*	*l*
No turn		⅛ turn		¼ turn		⅜ turn		½ turn		⅝ turn		¾ turn	
1	2	1	2	1	2	1	2	1	2	1	2	1	3
3	4	3	4	3	4	3	4	3	4	3	4	3	4
5	6	5	6	5	6	5	6	5	6	5	6	5	6
7	8	7	8	7	8	7	8	7	8	7	8	7	8
9	10	9	10	9	10	9	10	9	10	9	10	9	10
11	12	11	12	11	12	11	12	11	12	11	12	11	12
13	14	13	14	13	14	13	14	14	13	14	13	14	13
15	16	15	16	15	16	16	15	16	15	16	15	16	15
17	18	17	18	17	18	18	17	18	17	18	17	18	17
19	20	19	20	20	19	20	19	20	19	20	19	20	19
21	22	21	22	22	21	22	21	22	21	22	21	22	21
23	24	24	23	24	23	24	23	24	23	24	23	24	23
25	26	26	25	26	25	26	25	26	25	26	25	25	26
27	28	28	27	28	27	28	27	28	27	28	27	27	28
29	30	30	29	30	29	30	29	30	29	29	30	29	30
31	32	32	31	32	31	32	31	32	31	31	32	31	32
33	34	34	33	34	33	34	33	34	33	33	34	33	34
35	36	36	35	36	35	36	35	36	35	35	36	35	36

paternal. Thus 1 and 2 represented the two members of a pair of homologous factors, as did also 3 and 4, 5 and 6, etc., to 35 and 36, in every case the odd number representing the maternal, and the following even number the corresponding paternal factor.

I now secured the ends of the chromosomes so that the proper factors stood opposed to each other and began to twist the chromosomes.

It was at once apparent that if the twisting should not vary in the members of one species, coupling would be invariable, nor would the coupling be that representing either parent alone, but both, and the combination of factors would be always the same. This is shown in Table IX. In this table the vertical columns represent the gametes formed when a splitting follows the number of twists named at the top of the column. In each case there are two different combinations of gametes; one formed on the left and one on the right side of the split.

A study of the above table will show that if there be, for instance, no turn and the split falls between the two chromosomes all the factors in the gamete on the right will be maternal. If there be three eighths of a turn, the odd-numbered factors from 1 to 21 (maternal) and the even-numbered factors from 24 to 36 (paternal) will always be in the gamete on the right. And so in every case, if the amount of twist be constant, the factors present in any gamete will be constant. This will be true even if the factors are of different lengths, and the twisting not uniform for all parts of the same chromosome, if only the lack of uniformity be constant in all the members of the species.

Now, the nature of the twisting and the amount of variation that occurs can only be solved, if at all, by the cytologist. On the face of the question as so far presented it must be that variation in the twist occurs, or there would, on the basis of the theory here discussed, be no such thing as " independent mendelizing," but a constant coupling. My question is therefore, " What are the facts concerning this twisting? How uniform is it? " For I conceive it to be possible that if the twisting be nearly definite, coupling of certain factors would generally occur, and would depend not entirely on the nearness together of the factors, but on the amount of the twist, and on the side of the splitting

double chromosome on which any factor should be thrown by the twist. As Morgan points out, the chances of separation are not so great for factors which lie near each other as for those far from each other, though coupling would not necessarily imply that the factors should lie in one segment of the twisted chromosome. According to this idea the factors for wing length, as explained by Morgan in the paper in the *Journal of Experimental Zoölogy* for November, 1911, may not be closely approximated to those for eye color and body color.

Summary.

1. The factors for red and for white eye color and for normal and yellow body color in *Drosophila* have been the subjects of investigation in these experiments.

2. In the F_2 generation the factors for eye color and body color appear to a great extent associated in the same combinations that were present in the grandparents, the interchanging taking place only once to nearly eighty cases where there is no interchanging.

3. The absence in a fly of certain characters found in the normal wild fly, seem to render it less fertile, or at least such flies hatch in smaller numbers than do those in which such characters are present.

4. These facts are in accord with the principles and theories advanced by Morgan, and can be explained on a theory based on (1) the relative positions of factors in the chromosomes, (2) the twisting of homologous chromosomes about each other in gametogenesis, and (3) their subsequent splitting in one plane.

5. Further cytological evidence bearing on the above theory is much to be desired.

SIZE RELATIONSHIPS BETWEEN CONJUGANTS AND NON-CONJUGANTS IN BLEPHARISMA UNDULANS.

FLORENCE A. WATTERS.

It has been shown by Pearl ('07) that in *Paramœcium* cultures the conjugants differ from the non-conjugants in size and variability and that a correlation in size occurs between the two members of conjugating pairs. He has shown by statistical methods (1) that the conjugants are less variable in size than the non-conjugants, (2) that the conjugants show a smaller mean size than the non-conjugants, and (3) that a marked correlation in size exists between the members of the conjugating pairs, the smaller uniting with the smaller and the larger with the larger.

Jennings ('11) took up the problem of size relationships in *Paramœcium* going into discussion of the reasons for the conditions found, which he based on very careful observations of the conditions existing in his cultures. After careful measurement of a great many individuals he confirmed the results obtained by Pearl, *i. e.*, (1) that the conjugants are smaller than the non-conjugating population of a culture; (2) that they are less variable than the non-conjugants; and (3) that there is a marked correlation in size between the members of the pairs, so that on the whole larger individuals are found mated with larger, smaller individuals with smaller. It was with the idea of investigating the size relationships in another related form, that the present work on *Blepharisma undulans* was taken up. It will be possible here to give the facts of the size relationships only as they were found in material already mounted.

The individuals under consideration are from a pure-line culture isolated at Woods Hole on July 20, 1911, by Professor Calkins. The six lots with which I shall deal were killed by him at various times during October, 1911, by means of sublimate acetic, stained with Hoyer's picrocarmine, and mounted permanently in Canada balsam.

In measuring the individuals, a Leitz projectoscope was used

to throw a projection of each one upon the drawing table below. A permanent record of each one was obtained by drawing a line perpendicular to the long axis at each end. The figures given are the measurements of the projections rather than of the individuals themselves and are 192 times the actual size. Thus in the table:

	Longest, Mm.	Shortest, Mm.	Average, Mm.
Non-conjugants	42.5	13.5	27.67
Conjugants	30.0	15.5	20.12

the size of the projection rather than the actual size is given. Translated, these figures would be:

	Longest, μ.	Shortest, μ.	Average, μ.
Non-conjugants	221.35	70.31	144.11
Conjugants	156.25	78.12	104.79

But since it is the relative length rather than the absolute length which is of importance in this case, it has not seemed necessary to divide all the numbers given below by 192, and the figures denoting the size of the projection stand in the statistics which follow. For the sake of overcoming any element of personal error which may have entered into the measurements, all measurements falling half way between one mm. mark and the next are considered with those .5 mm. larger.

The results obtained by careful measurements of six lots of *Blepharisma undulans* are in accord with the results obtained by Pearl ('07) and Jennings ('11), *i. e.*, in *Blepharisma undulans* (1) the mean length of the conjugants is less than the mean length of the non-conjugants, (2) the variation in length is much less among the conjugants than among the non-conjugants, and (3) the correlation in size between the two members of a pair is distinctly marked, the larger mating with the larger and the smaller with the smaller. To take up first the difference in length between the conjugants and non-conjugants: Table I. shows that in all six lots the mean length of the conjugants is less than that of the non-conjugants.[1] Plate I. shows that the mean length of the

[1] The ordinates indicate the size of the individuals while the abscissas indicate the number of individuals; thus the position of the column indicates the size while the height of the column indicates the number of individuals.

conjugants of lot 1 is 21 units and that the number of individuals increases rather rapidly from the extremes, 17 and 30, toward the mean, 21; also that the mean length of the non-conjugants is 29 and that the number of individuals increases slowly at first and then more rapidly from the extremes 18 and 40 to the mean 29. The average lengths of the conjugants and non-conjugants is in accord with the mean length, being greater in the case of the non-conjugants and less in the case of the conjugants (Table I.).

Plate II. shows that the mean length of the conjugants of lot 2 is less than the mean length of the non-conjugants, the former being 22 and the latter 32. The number of individuals of the conjugants and non-conjugants increases from the extremes 18.5 and 28 in the case of the former, and 18.5 and 42.5 in the case of the latter, to the means 22 and 32. That the increase from extremes to means is much more gradual in lot 2 than in lot 1, is due to the fact that there are over twice as many individuals in lot 1 as in lot 2. The average length of the conjugants is less than that of the non-conjugants, as in lot 1, being 23.46 and 33.88 respectively.

TABLE I.

SIZE RELATIONS IN CONJUGANTS AND NON-CONJUGANTS.

Series.		I.	II.	III.	IV.	V.	VI.	Total.
No.	N.	390	148	551	47	47	198	1,381
	C.	160	30	292	20	20	28	550
Total length	N.	10,898	5,014.5	13,744	1,253	1,112.5	6,195	38,217
	C.	3,426.5	704	6,057.5	415.5	431	638.5	11,673
Average	N.	27.9	33.88	24.94	26.65	23.67	31.28	27.67
	C.	21.41	23.46	20.06	20.77	21.55	22.80	20.12
Longest	N.	40	42.5	33.5	34.5	34	40	42.5
	C.	30	28	27	25	25.5	27.5	30
Shortest	N.	18	18.5	13.5	18	18.5	16.5	13.5
	C.	16.5	18.5	16.5	15.5	18.5	19	15.5
Variations	N.	22	24	20	16.5	16.5	23.5	29
	C.	13.5	9.5	10.5	9.5	7	8.5	14.5
Mean	N.	29	32	25	29	27	32	27.5
	C.	21	22	21	20	22	23	21
Median	N.	29	30.5	23.25	26.25	26.75	28.25	28
	C.	23.25	23.25	21.25	20.25	22	23.25	22.75

N. indicates non-conjugants.
C. indicates conjugants.

Lot 3, Plate III., shows the same relative conditions as to mean and average length of conjugants and non-conjugants, the mean

length of the former being 21, and that of the latter 25. The averages are 20.06 and 24.94 respectively. The rapid increase in the number of individuals from the extremes 16.5 and 27, and 13.5 and 33.5 respectively is due to the larger number of individuals present in lot 3.

Lot 4, Plate IV., shows the same relative conditions as those found in the other lots, the mean lengths of conjugants and non-conjugants being 20 and 29 respectively, while the average lengths are 20.77 and 26.65. The small number of individuals occurring in lot 4 accounts for the lowness of the columns.

Lot 5, Plate IV., is like lot 4 in the number of individuals and in the shortness of the columns. There is greater irregularity of outline in lot 5 than in any other of the six lots, but the mean length of conjugants and non-conjugants is in accord with the mean length of the other lots, the former being 22 and the latter 27.

Lot 6, Plate IV., agrees with the rest in having the mean and average lengths of the conjugants smaller than those of the non-conjugants, the means being 23 and 32, and the averages 22.80 and 31.28 respectively. Thus in every one of the six lots, the mean and average have been less in the case of the conjugants than in that of the non-conjugants, the difference being from 5 to 10 units in the case of the means, and from 2 to 10 in the case of the averages. From all this we should expect to find that the mean and average of the total number of conjugants are less than those of the total number of non-conjugants. This is the case, the mean for the total number of conjugants being 21 and the average 20.12; and the mean for the total number of non-conjugants being 27 and 28 (or 27.5) and the average 27.67.

One interesting fact appears in Table I. and Plates I. to IV., which is shown best in Plates IV. and V., *i. e.*, the population including conjugants and non-conjugants shifts toward the left or right in the various lots. Plate V. shows this for the non-conjugants of lots 1, 2, and 3, the means being 29, 32, and 25, and the extremes 18 and 40, 19 and 43, and 14 and 34 respectively. The means of the non-conjugants of lots 4, 5, and 6, Plate IV., are 29, 27, and 32 respectively while the extremes are 18 and 35, 19 and 34, and 17 and 40. The conjugating populations do not show

this so well since they vary less than the non-conjugating populations.

This brings us to the consideration of the second of the three problems, *i. e.*, the variation of conjugants and non-conjugants. Table I. shows very clearly that the variation between the extremes in the case of the conjugants is much less than that in the case of the non-conjugants, being in the six lots:

13.5	9.5	10.5	9.5	7	8.5	in the former,
22	24	20	16.5	16.5	23.5	in the latter,

the variation ranging from nearly two times to nearly three times as great in the case of the non-conjugants. It is probable that some of the shortest non-conjugants are ex-conjugants, which would however not change the general results very appreciably.

Finally the third problem, as to coördination in size of the members of the same pair, appears. By plotting a graph using the length of the shorter of the two individuals as abscissa, and the longer as ordinate, the number of pairs of each combination which appeared was obtained. Then by adding diagonally, I obtained the number of pairs in which the individuals varied less than .5 mm. (or 2.2 microns, actual measurement), which varied .5 mm., 1 mm., 1.5 mm., etc., the following table resulting:

TABLE II.

CORRELATION IN SIZE OF MEMBERS OF CONJUGATING PAIRS.

	Pairs.
Both members equal in length	35
One member shorter by 0.5 mm.	57
One member shorter by 1.0 mm.	62
One member shorter by 1.5 mm.	50
One member shorter by 2.0 mm.	34
One member shorter by 2.5 mm.	17
One member shorter by 3.0 mm.	9
One member shorter by 3.5 mm.	8
One member shorter by 4.0 mm.	2
One member shorter by 4.5 mm.	2
One member shorter by 5.0 mm.	2
One member shorter by 5.5 mm.	1

No two members of the same pair show a greater variation than 5.5 mm., though conjugating individuals of different pairs show as great a variation as 14.5 mm.; and out of 279 pairs of con-

jugants only 7 pairs show a greater variation than 3.5 mm. This indicates a very definite correlation of size between the two members of the conjugating pairs.

The same conditions which Pearl ('07) and Jennings ('11) found to exist in *Paramœcium*, then, have been shown to exist in the case of *Blepharisma undulans*, *i. e.*, (1) the mean length of the conjugants is less than that of the non-conjugants, (2) the variation of the conjugants is less than that of the non-conjugants, and (3) there is a definite positive correlation in size between the members of the conjugating pairs, the larger uniting with the larger individuals and the smaller with the smaller.

REFERENCES.

Pearl, R.

'07 A Biometrical Study of Conjugation in *Paramœcium*. Biometrika, Vol. V., pp. 213–297.

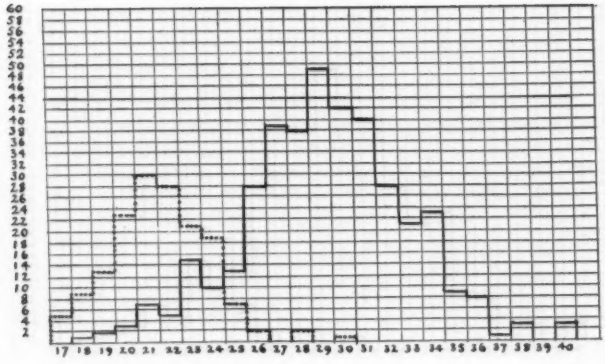
Jennings, H. S.

'11 Assortive Mating, Variability and Inheritance of Size in the Conjugation of *Paramœcium*. Jour. Exp. Zoöl., Vol. II., No. 1, July, 1911.

Explanation of Plates.

Plate I.

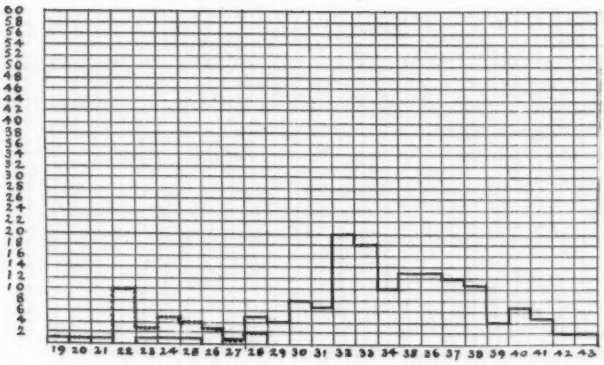
Lot 1. *Blepharisma undulans*. Abscissas: number of individuals. Ordinates: size of individuals. — non-conjugates. ..... conjugants.



FLORENCE A. WATTERS.

PLATE II.

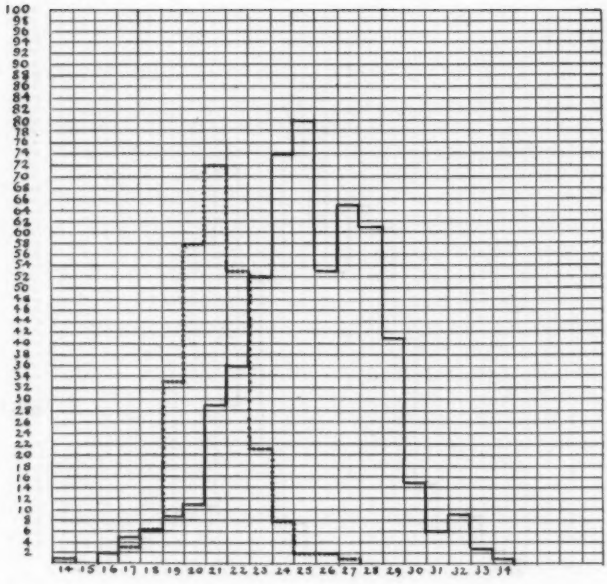
Lot 2. *Blepharisma undulans.* Abscissas: number of individuals. Ordinates: size of individuals. — non-conjugants. ..... conjugants.



FLORENCE A. WATTERS.

PLATE III.

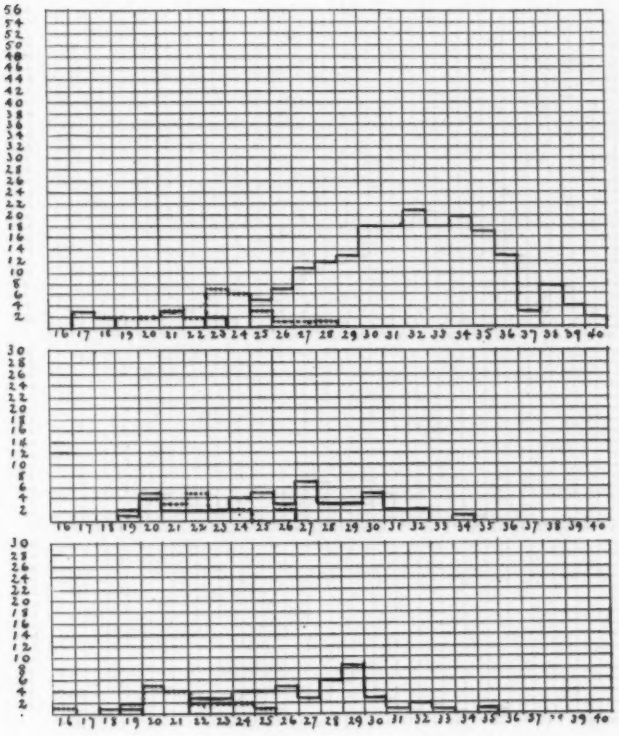
Lot 3. *Blepharisma undulans*. Abscissas: number of individuals. Ordinates: size of individuals. — non-conjugants. ..... conjugants.



FLORENCE A. WATTERS.

PLATE IV.

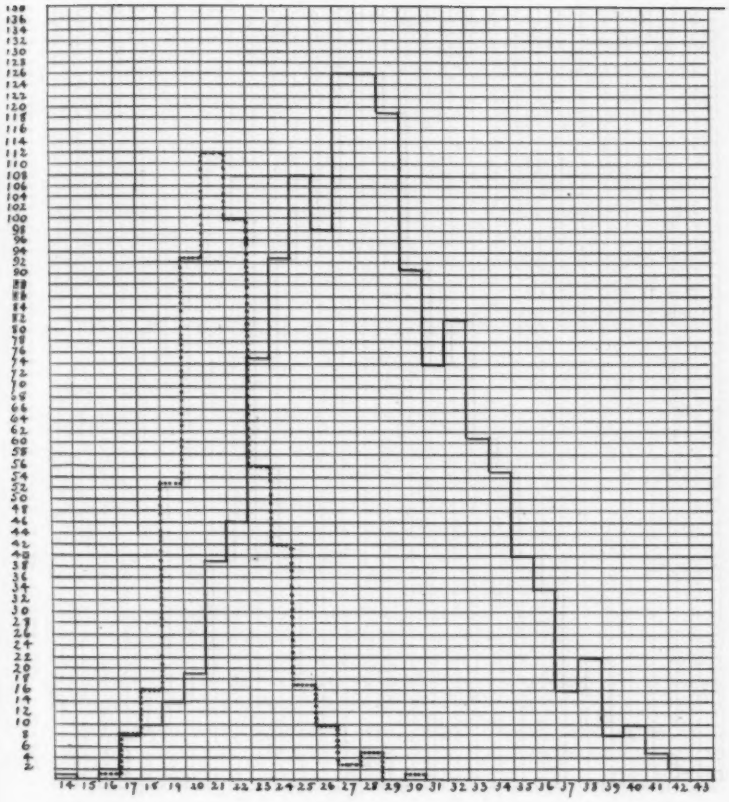
Lots 4, 5, 6. *Blepharisma undulans*. Abscissas: number of individuals. Ordinates: size of individuals. — non-conjugants. ..... conjugants.



FLORENCE A. WATTERS.

PLATE V.

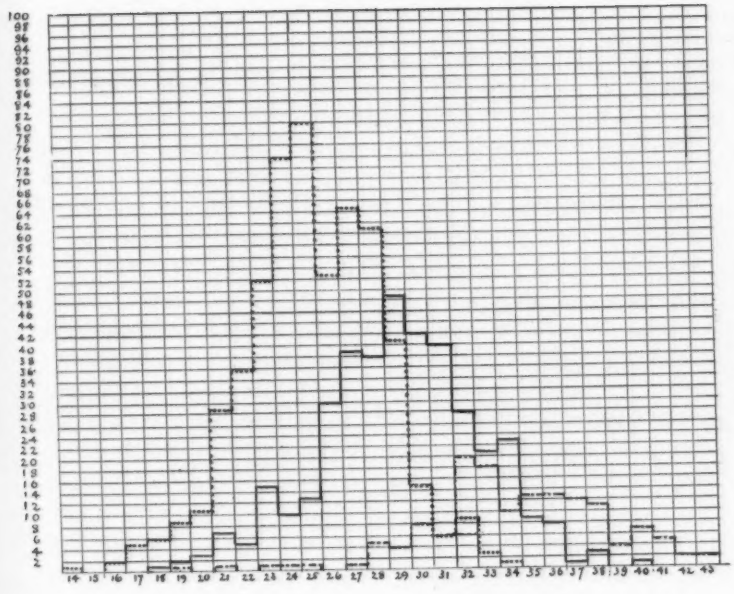
Lot "Total." *Blepharisma undulans*. Abscissas: number of individuals. Ordinates: size of individuals. — non-conjugants. ..... conjugants.



FIORENCE A. WATTERS.

PLATE VI.

Lots 1, 2, 3. *Blepharisma undulans*. Abscissas: number of individuals. Ordinates: size of individuals. — Lot 1. ..... Lot 2. — · — Lot 3.



FLORENCE A. WATTERS.

Mau